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EFFECT OF SULFONAMIDES AND THIOUREA DERIVATIVES ON HEART RATE AND ORGAN MORPHOLOGY

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A NUMBER OF sulfonamides and thiourea derivatives produce a goitrous hyperplasia of the thyroid accompanied by a marked decrease in basal metabolism (Richter and Clisby, 1942; MacKenzie and MacKenzie, 1943; Astwood, 1943). These actions have been attributed to a suppression of the thyroid secretions by the drugs. If this is the case, effects of thyroidectomy, such as bradycardia and atrophy of the kidney and other organs, should also be obtained with these drugs. It was therefore decided to investigate the action of representative sulfonamide and thiourea derivatives on the heart and other organs, as compared to that of thyroidectomy.

EXPERIMENTAL

In a preliminary experiment, 30 rats weighing from 90 to 130 gm. were divided into 3 groups. One group received a 1 per cent solution of thiourea as drinking water; another group was thyroidectomized; the third group was used for control. The experiment was continued for a period of 4 months (summer). Judging from the condition of the animals, this dose of thiourea was somewhat toxic. Heart rates were recorded electrocardiographically under nembutal anesthesia (Leblond and Hoff, 1944) at various time intervals after the beginning of the experiment (table 1). The heart rate was diminished by the treatment with thiourea as much as or even more than by thyroidectomy. At post-mortem, the animals in the thiourea group (table 2) showed goitrous thyroids with high epithelium and

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TABLE 1. COMPARATIVE EFFECTS OF THYROIDECTOMY AND THIOUREA TREATMENT ON THE HEART RATE OF THE RAT (MEASURED UNDER NEMBUTAL ANESTHESIA)

	Average heart rate at various time intervals after beginning of treatment		
	2 weeks	4 weeks	4 months
Controls	367 (340–400)	382 (368–408)	377 (353–402)
Thyroidectomized	324 (228–352)	258 (227–285)	253 (225–298)
1% thiourea in drinking water	313 (264–372)	243 (188–306)	218 (197–276)

Values in parentheses refer to extreme variations.

no colloid, enlarged chromophobic hypophyses without acidophilic cells, and small kidneys with accumulation of brown (wear-and-tear) pigment granules in the cells of the proximal convoluted tubules (fig. 1). Similar changes of the hypophysis and kidneys were observed in rats thyroidectomized for the same length of time.

TABLE 2. AVERAGE ORGAN WEIGHTS OF RATS TREATED WITH THIOUREA (GIVEN AS 1% SOLUTION IN DRINKING WATER FOR 4 MONTHS)

		Controls	Thiourea-treated
Average body weight at autopsy (gm.)		307 (255–365)	147 (120–200)
Hypophysis	(a)	11.5 (9.1–12.2)	10.4 (9.4–2.0)
	(b)	3.5 (2.9–3.1)	7.1 (5.0–8.7)
Thyroid	(a)	19.6 (14.5–26.6)	55.6 (42.0–98.6)
	(b)	6.2 (4.2–7.3)	37.2 (31.0–49.1)
Adrenals	(a)	47.7 (40–64)	23.3 (18–28)
	(b)	15.6 (13–21)	16.1 (12–20)
Kidneys	(a)	3219 (2215–4141)	1277 (1007–1703)
	(b)	1047 (720–1233)	869 (775–1005)
Heart	(a)	1040 (820–1249)	493 (337–664)
	(b)	339 (264–380)	339 (277–403)

a—mgm.; b) calculated in mg. per 100 gm. body weight.
Values in parentheses refer to extreme variations.

It was then decided to use other "anti-thyroid" compounds in doses which showed no apparent toxicity. Thiouracil[1] and sulfadiazine were given as saturated solutions in drinking water. Sul-

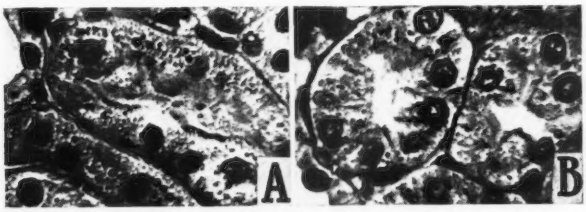

FIG. 1. Pigment granules of various sizes (A, small; B, large) in the cells of the proximal convoluted tubules of the kidney after treatment with thiourea.

fathiazole was mixed with the food (Purina Fox Chow) in the proportion of 1 per cent; this amount should produce a marked, although not maximal hyperplasia of the gland (Astwood, 1943).

TABLE 3. EFFECT OF SULFADIAZINE, SULFATHIAZOLE, AND THIOURACIL ON HEART AND ORGAN MORPHOLOGY IN COMPARISON WITH THE EFFECT OF THYROIDECTOMY

	Controls	Sulfadiazine* (0.1% water)	Sulfathiazole (1% food)	Thiouracil* (0.1% water)	Thyroid-ectomized
Quantity of drug ingested per rat (mg. daily)	—	25	158	31	—
Average body weight gain (gm.)	82	81	51	57	13
Average heart rate at end	405 (386–440)	427 (371–488)	326 (316–336)	303 (264–364)	275 (240–297)
Hypophysis:† Basophilic cells	n	(+)	+	++(+)	+++
Acidophilic cells	n	n	(−)	−	−−−
Average organ weights (mg.): Hypophysis	6.6 (4.7–9.1)	7.4 (5.4–9.1)	6.2 (5.5–6.8)	7.1 (6.0–7.7)	7.9 (6.4–9.0)
Thyroids	17.4 (13.0–21.7)	21.1 (15.5–24.0)	21.7 (17.0–25.3)	42.4 (20.6–57.3)	—
Adrenals	33.5 (24–40)	33.8 (22–44)	30.9 (27–33)	24.4 (17–32)	28.4 (24–31)
Kidneys	1848 (1819–1873)	1899 (1425–2209)	1584 (1432–1783)	1545 (1425–1719)	1223 (1095–1320)
Heart	724 (658–846)	698 (560–801)	534 (455–605)	616 (561–710)	513 (472–569)
Spleen	1505 (1032–2106)	1579 (1128–2302)	1461 (787–2069)	1244 (839–1464)	1188 (1073–1355)
Testicles	2462 (2206–2702)	2488 (1741–2878)	(2074–2629)	2342 (2093–2470)	2138 (1438–2538)
Liver	9214 (6927–10591)	9744 (7319–10769)	(8840–11792)	9771 (8485–11010)	(5866–7906)

Values in parentheses refer to extreme variations.
* Solution of sulfadiazine and thiouracil was not quite complete when amounts calculated to give a 0.1% solution were added to water.
† In estimating the hypophyseal changes the number of plus signs was used to grade the abundance of basophilic (thyroidectomy) cells; the number of minus signs is in relation to the scarcity of the acidophilic cells. The symbol n refers to a normal picture.

[1] For the thiouracil we are indebted to the Lederle Laboratories, Inc., Pearl River, N. Y.

Groups of 6 male rats weighing from 125 to 150 gm. were used for the assay of each substance. All animals were sacrificed 21 days after the beginning of the experiment. This experiment was carried out during the fall.

The results (table 3) showed that, except for the thyroidectomized control group, all the animals grew well during the experiment, although the animals treated with sulfathiazole or thiouracil gained less weight than the normal controls. The reduction of heart rates produced by thiouracil approached that obtained in thyroidectom-

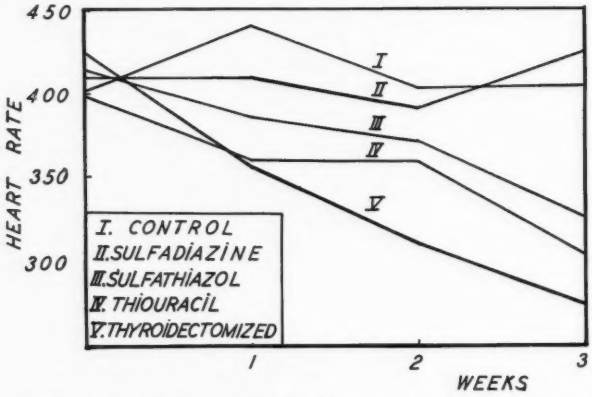

FIG. 2. Heart rates before and at weekly intervals after the beginning of treatment. (Nembutal anesthesia.)

ized animals; a slight reduction was effected by sulfathiazole; but sulfadiazine was without action on the heart (fig. 2). Examination of the thyroids revealed prominent goiters in the thiouracil group, and discrete hypertrophies with definite histological stimulation in the sulfathiazole group; in the sulfadiazine group, the effects on the thyroid were minimal. The hypophyses were examined by a method previously described (Leblond, 1944). The thyroidectomized animals showed the numerous enlarged basophils known as "thyroidectomy cells" and a complete absence of acidophils. In the drug-treated groups, the picture was that observed in cases of partial thyroidectomy. Thus, in the animals treated with thiouracil, there were fairly numerous thyroidectomy cells; some acidophils were present in all animals. Only three animals in the sulfadizaine group had any thyroidectomy cells. Finally, thyroidectomized animals showed a marked decrease in the weight of adrenals, kidneys, heart, and liver, and a slight decrease in the weight of lymphatic and genital organs. These effects were pronounced in the thiouracil group and slight in the sulfathiazole group, with the exception of the action on the liver, which showed an increase in weight. Sulfadiazine did not affect organ weights, except for a possible increase in liver weight.

DISCUSSION

The effects of treatment with thiourea, thiouracil, or sulfathiazole were similar to those of thyroidectomy, whether the heart rate, the hypophyseal picture, or the organ weights were considered. These results confirm that these drugs suppress the secretion of the thyroid.

It must be noted, however, that, with the doses used, the suppression of thyroid function was not complete except in the case of thiourea (1 per cent in drinking water). The thyroidectomy-like effects were rather marked with thiouracil (saturated solution in drinking water), moderate with sulfathiazole (1 per cent in food), and negligible with sulfadiazone (saturated solution in drinking water). These observations are not in agreement with the general impression that a complete suppression of thyroid function may be accomplished with the amount of thiouracil employed in these experiments (Astwood, 1943; Dempsey and Astwood, 1943).

With the exception of the increase in weight of the liver, the observed effects can be explained on the basis of a more or less complete deprivation of thyroid hormone. Liver atrophy due to thyroidectomy was not apparent in the drug-treated groups. On the contrary, there was a definite increase in liver weight as compared with the normal controls, indicating the presence of some direct effect of these drugs on the liver. It may be assumed that this effect overcame the weight reduction of the liver which should result from the inhibition of thyroid secretion.

SUMMARY

Thiourea given to rats as a 1 per cent solution in drinking water produced bradycardia, histologic changes in the hypophysis, and atrophy of kidneys, adrenals, and heart, equal in degree to the effects of complete thyroidectomy. Treatment of the rat with sulfathiazole as 1 per cent of the food or thiouracil as saturated solution in drinking water produced similar changes to a lesser degree. Sulfadiazine as saturated solution in drinking water had no significant influence on thyroid function. The only thyroidectomy effect which was not present in the drug-treated groups was the decrease in liver weight. The results are consistent with the assumption that, in the doses employed, thiourea completely suppresses thyroid function, while sulfathiazole and thiouracil inhibit it only partly.

REFERENCES

ASTWOOD, E. B.: *J. Pharmacol. & Exper. Therap.* 78: 79. 1943.
DEMPSEY, E. W., AND ASTWOOD, E. B.: *Endocrinology* 32: 509. 1943.
LEBLOND, C. P.: *Rev. Canad. Biol.* (In press.)
LEBLOND, C. P., AND H. E. HOFF: *Am. J. Physiol.* 141: 32. 1944.
MACKENZIE, C. G., AND MACKENZIE, J. B.: *Endocrinology* 32: 185. 1943.
RICHTER, C. P., AND CLISBY, K. H.: *Arch. Path.* 33: 46. 1942.

RESPONSE OF ADRENALECTOMIZED-HYPOPHYSECTOMIZED RATS TO THE PITUITARY GROWTH HORMONE[1]

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IT IS WELL known that adrenalectomy in young animals entails interference with growth. Grollman (1936) has indeed proposed the re-establishment of growth by administration of adrenocortical extracts as a satisfactory test for the presence of the vitally necessary adrenocortical hormone. It can, however, be shown that the interference with growth caused by adrenalectomy is due to disturbances in the electrolyte metabolism, because adrenalectomized rats grow normally when given 1% NaCl as drinking water. In the present paper, the attempt has been made to see whether the re-invocation of growth by administration of growth hormone to hypophysectomized rats is influenced by concurrent adrenalectomy. Since it is known that adrenal secretion in excess actually impedes growth, it was considered entirely possible that animals subjected to adrenalectomy as well as hypophysectomy might be even more sensitive to growth hormone than the hypophysectomized rat.

THE ADRENALECTOMIZED-HYPOPHYSECTOMIZED RAT

The adrenalectomized-hypophysectomized rat used in this study is an extremely fragile experimental animal, as already remarked upon by experimenters who have attempted its use (e.g., Levie and Uyldert, 1939). The delicacy of the hypophysectomized rat (poor appetite, poor body tone, susceptibility to infection) is also well known, but the percentage survival is high enough to make it a thoroughly practicable experimental animal. Rats only subjected to adrenalectomy, if maintained on 1% NaCl drinking water, are so nearly normal as to be scarcely distinguishable from normal animals

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in body tone, appetite, growth rate and resistance to infections. When, however, the deficiencies due to the two operations are superimposed, the resultant animal has a very poor survival. Most of the animals retained as controls (without hormone therapy) died by the fifteenth day after the second operation (hypophysectomy). Multiple gastric ulcers[2] were common in these rats. Because of these characteristics of the experimental animal it seems important to devote some time to discussing the conditions under which these animals were prepared and to describe efforts made to prolong their life span.

Male rats, 27 to 35 days of age, were adrenalectomized. After operation they were maintained on drinking water containing 1% NaCl. The diet was Diet XIV[3] supplemented by lettuce 3 times weekly. A period of recovery was allowed and hypophysectomy was performed 7 to 10 days later, when the rats were 39 to 42 days old. As is customary with all hypophysectomized rats, they received 1 cc. of 20% glucose intraperitoneally daily, and the diet was supplemented daily by Diet I[4] made into a wet mash. The rats were kept in a room maintained at 75–78° F., and in cold weather extra heaters were put on their cages. The rats were kept on shavings, 3 or 4 to a cage to aid in maintaining their body temperature. It was thought that survival might be improved by diluting the glucose to 10% and making it up in normal saline (0.85% NaCl). This solution was given intraperitoneally twice daily in doses of 0.5 to 1 cc. each. Still later, 5% sucrose was added to the drinking fluid in an attempt to support the animals.

Only animals which were shown to have had the two types of endocrine organs completely removed were included in the accompanying tables. The hypophysectomy was checked as usual by examination of the sella turcica at autopsy with a binocular (at 10× magnification). Any fragments suspected of being pituitary tissue were fixed, sectioned, stained and examined microscopically. The site of the adrenals was also searched at autopsy and any unidentifiable tissue sectioned and examined.

Results from a series of experiments clearly indicate that the supplements of salt and carbohydrate were not successful in prolonging the survival period of adrenalectomized-hypophysectomized controls. Of 53 rats subjected to the double operation, and retained as controls of experimental groups, 11 or 20% survived 15 days. Rats hypophysectomized at the same age, 40 days, and maintained

[2] Gastric ulcers have not been noted in adrenalectomized rats and are infrequent in hypophysectomized rats, but very common in adrenalectomized-hypophysectomized rats. Freud, Luwisch and Oestreicher have noted their occurrence after adrenectomy in the dog.

[3] Diet XIV consists of 68.5% wheat, 5% casein, 10% fish meal, 10% alfalfa, 1.5% NaCl, 5% fish oil.

[4] Diet I, McCollum's Diet I modified slightly, consists of wheat 67.5%, casein 15%, whole milk powder 10%, NaCl 1%, $CaCO_3$ 15%, homogenized vegetable oil 5%. A concentrate of fish oil was added so that each gram of the mixture contained 1 to 2 chick units of vitamin D, and 11 to 12 USP units of vitamin A.

TABLE 1. RESPONSE OF ADRENALECTOMIZED-HYPOPHYSECTOMIZED RATS TO GROWTH HORMONE*

Series	Daily dose IP	Body weight					Body length		Width of epiphyseal cartilage‡
		Adrx.	Day after hyph.†	Autopsy	Gain in 10 da.	Gain in 16 da.	At autopsy	Increase in 15 days	
	mg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cm.*	*micra*
	0.4	84	104	151	26	59	33.2		
		60	107	123	13	26	33.4		
		74	102	161	35	59	33.9		
I		70	104	164	23	60	33.7		
		(62)	(90)	—	died day 4		—		
		(78)	(100)	—	died day 6		—		
Adrx. da. 27		(71)	(101)	—	died day 7		—		
Hyph. da. 38									
Aut. da. 55	Av. (4)	72	104	149	32	51	33.5		
		(68)	(94)	—	died day 1		—		
		(63)	(90)	—	died day 2		—		
	Control	(68)	(87)	—	died day 2		—		
		(72)	(103)	—	died day 8		—		
	0.2	101	114	146	15	32	34.7		457
		89	96	111	6	5	33.2		—
		90	99	133	27	34	33.6		416
		97	116	149	26	33	34.5		368
		106	120	144	20	24	34.4		358
		101	114	145	32	31	33.9		405
II		101	121	169	27	43	35.1		422
	Av. (7)	98	111	142	22	29	34.2		404
Adrx. da. 33		102	106	91	+2	−15	30.8		222
Hyph. da. 39		92	109	101	−5	− 8	28.8		268
Aut. da. 57	Control	97	112	118	+4	+ 6	27.0		313
		92	109	96	−9	−13	27.8		258
		84	100	81	−8	−16	28.0		216
		(94)	(88)	—	died day 5		—		—
		(83)	(100)	—	died day 8		—		—
	Av. (5)	93	107	97	−3	− 9	28.5		255
		110	138	175	18	37	35.5	2.6	
		99	122	148	20	26	34.0	2.9	
		110	134	153	6	19	32.4	2.2	
III		91	116	134	14	18	31.0	2.5	
	0.2 (da. 1–5)	92	116	113	6	−3	31.7	2.1	
	0.3 (da. 6–15)	105	132	158	26	26	34.8	3.1	
Adrx. da. 35		96	122	161	26	39	34.2	3.5	
Hyph. da. 41									
Aut. da. 56		(111)	(140)	—	died day 3				
		(110)	(130)	—	died day 6				
	Av. (7)	100	126	163	17	23	33.4	2.9	

* The potency of the growth hormone preparations used is defined in the text.

† Series I and II. In series III the rats were weighed on the day of hypophysectomy and so do not show the weight loss usually incurred during the 24 hours following operation.

‡ Measurements made from median sagittal section of proximal epiphysis of tibia, stained with hematoxy in and eosin.

under comparable conditions,[5] almost all survived for 15 days, 90 out of 94 or 96%.

RESPONSE OF ADRENALECTOMIZED-HYPOPHYSECTOMIZED RATS TO GROWTH HORMONE

Three experiments are recorded in table 1, showing the responsiveness of the adrenalectomized-hypophysectomized rats to growth hormone. There are slight differences in the age at which the male rats were adrenalectomized, 27 to 35 days of age. The period of recovery before the second operation was also varied, from 6 to 11 days, so that all animals were approximately the same age at

[5] The conditions for maintenance of hypophysectomized rats were: dry diet XIV supplemented by wet diet I and lettuce, colony 75–78° F, heaters on cages in cold weather; water to drink, 20% glucose intraperitoneally in 1 cc. doses 4 times weekly.

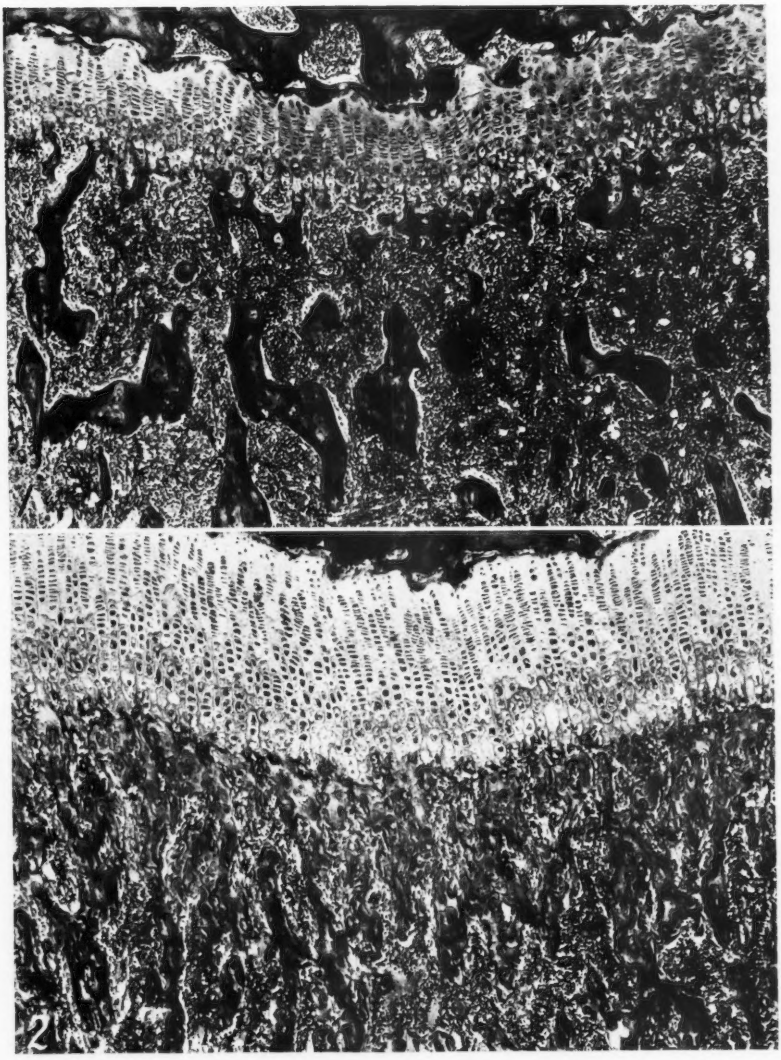

FIG. 1. Proximal epiphyseal region of tibia of control adrenalectomized-hypophysectomized male rat (adrenalectomized at 33 days of age, hypophysectomized at 40 days, autopsied 16 days after second operation; supported by 0.85% NaCl with 10% glucose, intraperitoneally, and 1% NaCl plus 5% sucrose in drinking water; no hormonal therapy). Spec. 7999. Pl. 7949.

FIG. 2. Proximal epiphyseal region of tibia of growth hormone injected adrenalectomized-hypophysectomized rat (male rat adrenalectomized at 34 days of age, hypophysectomized at 41 days; supported as in control by salt and carbohydrate; received 0.2 mg. of growth hormone daily for 15 days beginning the day after hypophysectomy; autopsied 16 days after second operation). Spec. 8011. Pl. 7950.

from Diet I was added to the food after hypophysectomy. In all series 10% glucose in 0.85% NaCl was given intraperitoneally twice daily in 0.75 cc. doses. The 1% NaCl drinking water given from the

TABLE 2. COMPARISON OF THE RESPONSE TO GROWTH HORMONE* OF ADRENALECTOMIZED-HYPOPHYSECTOMIZED RATS WITH THAT OF HYPOPHYSECTOMIZED RATS

Operation	Daily dose IP	Body weight					Body length		Tibia	
		Adrx. 34 da.	Day after hyph. 43 da.	Autopsy 58 da.	Gain in 10 da.	Gain in 15 da.	Autopsy	Increase in 15 da.	Length	Width of cartilage†
	mg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cm.*	*cm.*	*cm.*	*micra*
Adrx. and Hyph.	0.5	105	121	181	44	60	35.0	3.5	3.49	332
		129	163	220	43	57	36.7	3.4	3.70	310
		98	118	125	22	7 (sick)	32.5	2.6	3.39	262
		(97)	(113)	(115)	12	died day 15	(33.5)	(3.2)	(3.43)	(187)
		(103)	(123)	(103)	(− 2)	died day 15				
		(104)	(117)			died day 5				
		(92)	(125)			died day 8				
		(83)	(100)			died day 12				
	Av. (3)	111	134	175	30 (4)	41	34.7	3.2	3.53	301
	Control	96	115	108	− 2	− 7	29.7	1.6	3.13	141
		(92)	(110)		—	died day 7				
		(98)	(120)		—	died day 8				
		(94)	(116)		—	died day 9				
		(84)	(106)		− 6	died day 10				
		(107)	(120)		+ 1	died day 11				
		(106)	(125)		+ 2	died day 13				
		(103)	(118)		−10	died day 14				
Hyph.	0.5		117	163	31	46	32.2	3.5	3.10	373
			128	179	31	51	34.4	3.2	3.51	366
			113	155	27	42	33.8	4.0	3.40	306
			126	163	20	37	33.6	2.7	3.40	299
			120	172	33	52	34.2	3.5	3.41	318
			136	164	21	28	34.4	3.3	3.43	208
	Av. (6)		123	166	27	43	33.8	3.7	3.38	312
	Control		129	110	−11	−19	32.3	1.2	3.21	117
			116	108	− 9	− 8	28.9	0.9	3.02	117
			106	113	9	7	31.3	0.7	3.10	124
			124	128	5	4	31.2	1.1	3.20	138
			115	117	0	2	30.5	1.8	3.10	141
	Av. (5)		118	115	− 1	− 3	30.8	1.1	3.13	127

* The growth hormone preparations were the same as that used in Series III, table 2.
† Proximal epiphyseal cartilage of tibia; measurements made on split tibia stained with silver nitrate.

hypophysectomy and autopsy. As in the controls, the wet mash made time of adrenalectomy was supplemented in Series II and III by 5% sucrose. Injections of growth hormone were begun in half of the rats of Series I and II (7 per group)[6] the day after hypophysectomy, the other half of the rats (7 per group) being retained as controls. In Series III nine rats were injected with growth hormone, none being retained as controls. The growth hormone[7] was a highly purified

[6] Discrepancies between numbers of rats cited in the text as being placed in the experiment and the number appearing in the tables, are due to elimination of animals in which either operation was incomplete.

[7] The hormone preparation used in Series I and II (PH XXIX 3b) contained $70[GU]^{15}_{HR}$ (growth units per mg. based on the injection of hypophysectomized rats for a 15 day period, see Marx, Simpson and Evans, 1943, p. 78, Table 1, footnote). The preparation, when injected into hypophysectomized immature female rats, 14 days postoperative, in a total dose of 1.4 mg. over 4 days, caused no ovarian nor adrenal response, and had a minimal effect on the thyroid. This minimal effect was also seen in the thyroids of the doubly operated rats at a daily dose of 0.4 mg. Series I, but not

product. It was given as a single intraperitoneal injection daily for 15 days in doses ranging from 0.2 to 0.4 mg. as cited in table 1.

It will be noted from table 1 that not only body weight increment but also skeletal growth was obtained. At the end of the 15 day injection period, the body length of injected rats in Series II averaged 5.7 cm. more than that of the surviving controls. The proximal epiphyseal cartilage of the tibia was practically twice as wide in injected as in uninjected rats.

The chondrogenic and osteogenic activity aroused in these rats by growth hormone is also shown in Plate I, figures 1 and 2. Figure 1 shows the proximal epiphyseal region of the tibia of an untreated adrenalectomized-hypophysectomized rat at the end of the 15 day experimental period. The narrow epiphyseal disc composed of a few layers of small cells should be noted. Although a few vacuolated cartilage cells were present, the number of capillaries directed against the cartilage was small. Osteogenesis had ceased and a much reduced amount of trabecular bone was present. In most cases the cartilage abutted directly against the marrow, in some places being partially sealed off from the marrow by bone. In figure 2 it will be seen that when growth hormone was administered during the 15 days the columns of cartilage cells were much higher, many layers being present. The cartilage cells were larger than in the controls, throughout at least the lower half of the cartilaginous disc. The vacuolated cartilage cells at the ends of the columns were opened in every instance by capillary tufts. Even more striking was the great acceleration of osteogenesis evidenced by the abundant medullary bony trabeculae next the ossification zone.

RELATIVE SENSITIVITY OF THE HYPOPHYSECTOMIZED AND THE ADRENALECTOMIZED-HYPOPHYSECTOMIZED RAT TO GROWTH HORMONE

As the hypophysectomized rat synthesizes enough adrenal-cortical steroids to maintain life, even showing relatively slight disturbance in carbohydrate and less disturbance in salt metabolism, it was thought possible that this animal might still be able to supply enough adrenal cortical hormone to antagonize growth hormone to some extent. If this were true, the hypophysectomized rat might be less sensitive to growth hormone than the adrenalectomized-hypophysectomized rat. The two types of experimental animals were therefore injected for the same length of time with the same dose of growth hormone (table 2). Male rats were adrenalectomized at 34 days

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at 0.2 mg. Series II. The preparation used in Series III, pH XXXV, contained 20$[GU]^{101}_{HR}$. Tested for impurity in immature hypophysectomized rats, 14 days postoperative, in a total dose of 5 mg. in 4 days, it caused no ovarian nor adrenal response and had a barely detectable effect on the thyroid. This same barely detectable but definite response was seen in the thyroids of the doubly operated rats receiving 0.2 to 0.3 mg. daily, Series III.

of age. From the time of operation they received 1% NaCl as drinking water. They were hypophysectomized at 42 days of age and from this time 5% sucrose was added to the saline drinking fluid. In addition, they received 0.5 cc. of 10% glucose in physiological saline, intraperitoneally, twice daily. The rats having had the double operation were divided into two groups of 9 rats each, the hypophysectomized rats were divided into two groups of 6 rats each. One group of doubly operated and one group of hypophysectomized rats received the growth hormone. It was given once daily in 0.5 mg. doses intraperitoneally for a 15 day period beginning the day after hypophysectomy.

As will be seen in table 2, the responsiveness of the doubly operated rat was similar to that of the hypophysectomized rat; no increased sensitivity was established. Levie and Uyldert in 1939 investigated the responsiveness of the adrenalectomized-hypophysectomized rat to growth hormone and found it equal to that of the hypophysectomized rat.

The great improvement in survival rate of the adrenalectomized-hypophysectomized rats when injected with growth hormone should be noted of thirty-one rats which had been doubly operated and injected with growth hormone from the day after hypophysectomy, 23 survived to the fifteenth day, a survival of 77 per cent. Rats which did not receive hormone had a 20% survival (11 of 53 rats survived to 15 days).

SUMMARY

Adrenalectomized-hypophysectomized rats supported by salt, and receiving carbohydrate supplementary to the diet by injection, had a conspicuously higher mortality than is the case with hypophysectomized rats. Pituitary growth hormone administered to these rats substantially decreased this mortality. Adrenalectomized-hypophysectomized rats showed the same sensitivity to pituitary growth hormone as characterizes hypophysectomized rats; reference is made to increased body weight, skeletal dimensions and thickness of the epiphyseal cartilage.

REFERENCES

FREUD, J., D. LUWISCH AND F. OESTREICHER: *Brit. M. J.* 1: 1216. 1935.

GROLLMAN, A.: The Adrenals, Williams and Wilkins, 1936.

LEVIE, L. H., AND I. E. UYLDERT: *Acta Brevia Neerlandica de Physiologica, Pharmacologica, Microbiologica E. A.* 9: 121. 1939.

MARX, W., M. E. SIMPSON AND H. M. EVANS: *J. Biol. Chem.* 147: 77. 1943.

STUDIES ON THE MECHANISM OF ALLOXAN DIABETES

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IN APRIL 1943 Shaw Dunn, Sheehan and McLetchie announced their discovery that alloxan, the ureide of mesoxalic acid, causes necrosis of the pancreatic islet cells. In the meantime, the diabetogenic action of alloxan has been demonstrated almost simultaneously by Dunn and his associates in Glasgow (1943), by Bailey and Bailey in Boston (1943), and by Goldner and Gomori in Chicago (1943). Alloxan diabetes has been produced in rats, rabbits, dogs, monkeys, and cats (Goldner and Gomori, 1944). Anatomically, this diabetes is characterized by the selective necrosis of the beta cells in the islets of Langerhans.

Prior to the establishment of the diabetes, the blood sugar of alloxan treated animals shows a characteristic triphasic reaction, which was first observed by Jacobs in 1937. An immediate hyperglycemia which reaches its peak within two or three hours, is followed by a severe, often fatal hypoglycemia, which after a duration of several hours yields to the final hyperglycemia (Fig. 1).

The present paper is concerned with the mechanism of the diabetogenic action of alloxan and with the significance of the initial fluctuation of the blood sugar level. It will be shown also that the insulin content of the pancreas of dogs with alloxan diabetes is markedly reduced and that the diabetogenic action is a specific property of alloxan which cannot be found in related compounds or in certain other oxidising agents.

The effect of insulin and phlorhizin on the development of alloxan diabetes. In other forms of experimental diabetes, after partial pancreatectomy as well as after treatment with anterior pituitary extract (APE), the islet cell degeneration is preceded by hyperglycemia (Houssay et al., 1942, Lukens, Dohan and Wolcott, 1943). This hyperglycemia apparently puts an overstrain on the pancreas and

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leads first to exhaustion of the islet cells which may be reversible, and later to irreversible degeneration. If the initial rise of the blood sugar is prevented by starvation or by treatment with insulin or with phlorhizin, the islet cells can be protected against degeneration, and no diabetes will develop (Young 1939, Campbell, Haist, Ham and Best, 1940, Lukens, Dohan and Wolcott, 1943). Such procedures, however, do not protect alloxan-treated animals and diabetes ensues

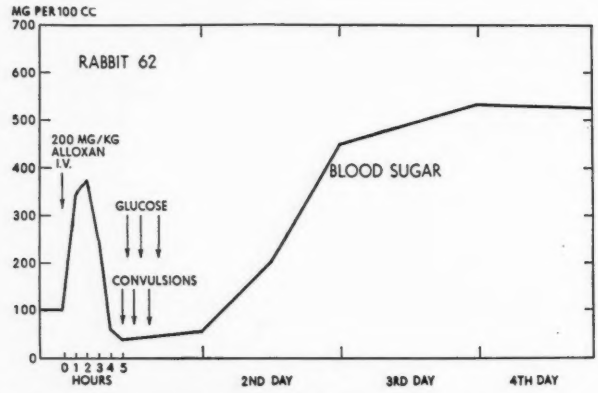

FIG. 1. The triphasic reaction of the blood sugar of the rabbit to a diabetogenic dose of alloxan. Initial hyperglycemia, transitory hypoglycemia, relieved by glucose injections and final diabetic hyperglycemia.

in any case. This direct diabetogenic effect of alloxan is demonstrated by the following experiments.

Experiment I. Figure 2 shows the course of the blood sugar level, urinary output and glycosuria of an alloxan-treated dog, in which the early phase of the hyperglycemia was prevented by insulin treatment. Four hours before the diabetogenic dose of alloxan (50 mg. per kg). was injected intravenously, this dog was given a first dose of 6 units protamine zinc insulin (PZI). During the following 4 days 8 units of PZI were given daily; the fasting blood sugar remained within the normal range, the urine was sugar free, except on the second day when 3 gm were excreted. As soon as insulin treatment was discontinued, a marked hyperglycemia and glycosuria became evident. Insulin had not prevented the development of diabetes. After a few days insulin treatment was renewed. Again 8 units PZI were sufficient for diabetic control, there was no change in the sensitivity to insulin. When after a week insulin was stopped, the diabetes again became evident, and a biopsy of the pancreas revealed islet cell degeneration of the same extent as in untreated animals.

Experiment II. Two dogs were given daily injections of 0.2 gm phlorhizin subcutaneously for a period of 10 days (figure 3). Marked

glycosuria resulted; the blood sugar remained at the normal level. On the ninth day a diabetogenic dose of alloxan was injected intravenously. In both dogs marked hyperglycemia developed, and biopsies from the pancreas showed the characteristic islet cell degeneration In dog 203 a first biopsy was taken on the 7th day of phlorhizin treatment and showed an entirely normal structure of the islets.

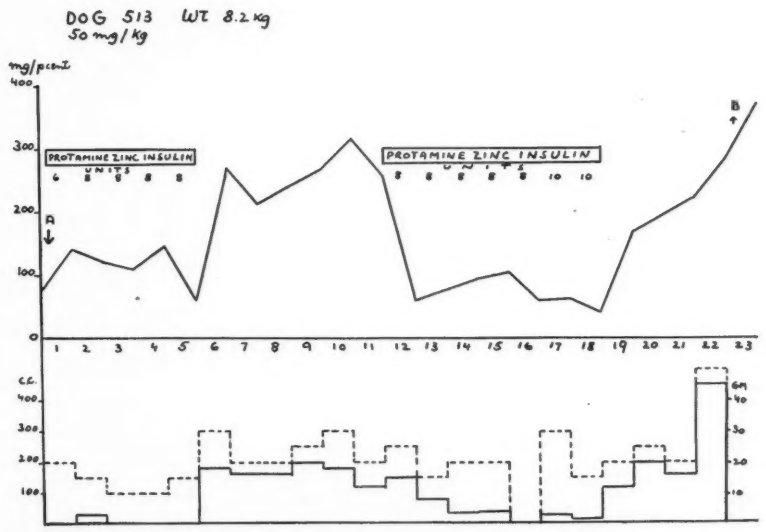

FIG. 2. The effect of insulin on the development of alloxan diabetes in the dog. Six units Protamine zinc insulin (PZI) were given 4 hours before 50 mg. per kg. alloxan were injected intravenously. Daily insulin injections of 8 units PZI were given for the next four days and resumed for the period between the 12th and 17th day. The effect on the blood sugar was the same during both periods Ten units PZI produced hypoglycemic levels (17th and 18th day). Blood sugar level and amount of daily urine and glucose excretion are shown. B indicates biopsy.

Experiment III. Six rabbits were injected simultaneously with insulin and alloxan. Both injections were given intravenously. The dose of insulin was 1 unit per kg., the dose of alloxan 150 and 200 mg. per kg. In the first 3 animals insulin and alloxan were injected into different veins; in the other 3 animals a mixture of alloxan and insulin was given. In none of the animals did the initial hyperglycemia develop. All of them went into the hypoglycemic phase, which in two instances was fatal. Biopsies during this phase revealed early degenerative changes in the pancreatic islets. The surviving 4 rabbits developed diabetes (table 1). Insulin had prevented the hyperglycemia but the degeneration of the islet cells occurred and diabetes developed finally.

Significant differences thus appear between the diabetogenic mechanisms of alloxan and APE. The early hyperglycemia after alloxan is not a causal factor in the development of diabetes, since its prevention does not protect against islet cell degeneration (experiments 1–3); the sustained hyperglycemia which develops after the initial fluctuations of the blood sugar is the result of the islet cell de-

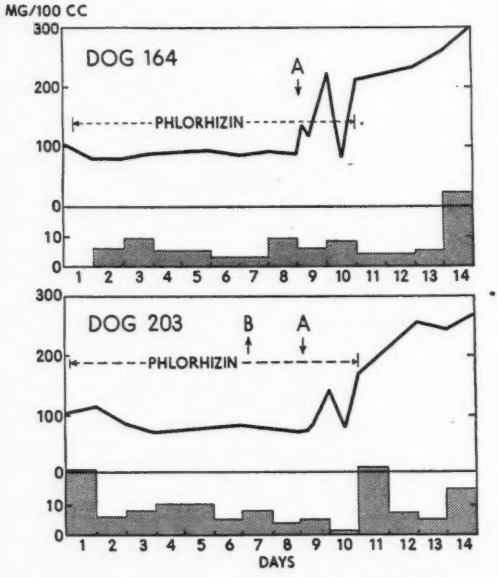

FIG. 3. Development of diabetic hyperglycemia in two dogs, which were treated with daily injections of 0.2 gm. phlorhizin, preceding and following the injection of a diabetogenic dose of alloxan (A).

generation (experiment 3). Diabetes can be produced by a single dose of alloxan, whereas only prolonged treatment with increasing amounts of anterior pituitary extract is diabetogenic (experiments 1–3). APE diabetes has an initial period of refractoriness to insulin; alloxan diabetes in its early phase is insulin sensitive; this is demonstrated in experiment 1, but has been proven in several other dogs by insulin sensitivity tests which were performed periodically.

The initial fluctuations of the blood sugar. What is the cause of the initial alloxan hyperglycemia? The fact that it is prevented by insulin argues against the possibility that the first effect of alloxan may be a transitory inhibition of insulin. This possibility was ruled out further when we found that insulin was not inactivated by alloxan in vitro. It seemed, therefore, likely that the initial hyperglycemia is not due to lack of insulin, but to mobilization of extra glucose. This may be

brought on through increased glycogenolysis from the liver under the influence of epinephrine. Young and his associates (1943) were able to reproduce the initial alloxan blood sugar curve by the injection of adrenalin and insulin into normal rabbits.

We subjected this hypothesis to experimental test by the injection of alloxan into functionally or anatomically adrenalectomized rabbits.

TABLE 1. EFFECT OF INTRAVENOUS INJECTION OF ALLOXAN AND INSULIN ON THE BLOOD SUGAR OF THE RABBIT

	Blood sugar level								
	Fasting	1	2	3	4	5	6	24	48
		Hours after injection							
Alloxan control (Average)	110	295	340	270	115	52	34	120	316
R 38	111	79	69	69	60	—	—	438	—
R 18	117	65	64	93	84	—	43	—	507
R 52	122	139	141	135	—	—	45	—	532
R 2	82	40	32	—	—	—	—	—	—
R 16	115	83	61	85	—	37	—	—	—
R 4	107	70	53	75	—	—	55	113	279
Insulin control	121	30	36	39	55	90	115	—	—

TABLE 2. EFFECT OF INTRAVENOUS INJECTION OF ALLOXAN ON THE BLOOD SUGAR LEVEL OF THE ADRENALECTOMIZED RABBIT

	Blood sugar level								
	Fasting	1	2	3	4	5	6	24	48
		Hours after injection							
Alloxan control (Average)	110	295	340	270	115	52	34	120	316
AE 81	105	36	29	—	—	—			
AE 22	117	51	—	—	—	—			
AE 86	98	35	—	—	—	—			
AE 42	96	107	119	121	—	—	32	Died in typical hypoglycemic convulsions	
AE 25	114	145	148	41	—	—			
AE 41	109	117	128	—	30	—			
Insulin control	121	30	36	39	55	90	115	—	—

Experiment IV. The adrenals of 3 rabbits were removed and in 3 others the medulla was destroyed by intramedullary injection of formalin. The adrenals were removed one at a time, in two operations 24 to 48 hours apart. The right and left adrenals were injected with formalin several days apart. A diabetogenic dose of alloxan was given immediately after the removal of the second adrenal, or two days after the second injection of formalin. In no instance did any marked intial hyperglycemia develop. All animals developed severe fatal hypoglycemia (table 2). In 3 animals the hypoglycemia occurred about two hours earlier than in intact rabbits; in these the lack of adrenal counter-regulation probably enhanced the fall of the blood

sugar. The formalin-injected adrenals were examined at autopsy in order to check the extent of damage. In all animals the medulla was destroyed either completely or to a considerable extent, and the damage extended to the inner layers of the adrenal cortex.

It can be concluded from this experiment that adrenal stimulation of glycogenolysis is involved in the production of the initial alloxan hyperglycemia. The recent findings of Hard and Carr (1944) that histological changes in the adrenal medulla occur immediately after the injection of alloxan, also support this conclusion.

The second phase of the reaction of the blood sugar to alloxan, the *hypoglycemia*, was originally interpreted as resulting from an insulin-like effect of alloxan itself (Jacobs, 1937). Were this true, the blood sugar of depancreatectomized animals should also fall in response to alloxan.

Experiment V. Diabetogenic doses of alloxan were given to depancreatized dogs and also to rabbits which previously had been

TABLE 3. INSULIN ASSAYS IN THE PANCREAS OF ALLOXIAN DIABETIC DOGS

Dog no.	Duration of diabetes	Insulin content of pancreas
513	18 days	Less than 0.7 units per gram
311	36 days	Less than 0.5 units per gram
67	60 days	Approx. 0.6 units per gram

rendered diabetic by alloxan. No lowering of the blood sugar level was noted in either group. The presence of a normally functioning pancreas therefore seems to be necessary for the occurrence of the hypoglycemic reaction, and it would appear that alloxan changes glucose metabolism through its effect on the pancreatic islet cells. This conclusion is further substantiated by the work of Corkill, Fantl and Nelson (1944), who found that alloxan did not influence the blood sugar level of the eviscerated cat.

If the hypoglycemia is brought about by the effect of alloxan upon the pancreas, it might be caused either by the stimulation of islet cells or by the release of stored insulin from the degenerating cells. The first view put forth by Dunn and his co-workers (1943) suggests that increased islet cell activity and over-production of insulin may overstrain the cells and cause their subsequent failure and degeneration. The experimental evidence, however, seems to favor the second possibility, and we believe with Young and his co-workers (1944) that the hypoglycemia is the first sign, rather than the cause of the developing necrosis. We base our belief on the following evidence:

1. Beta cell degeneration precedes the hypoglycemia. In histological studies on rabbits and dogs we have found that degeneration of the beta cells is demonstrable as early as one hour after injection of alloxan. It precedes the hypoglycemia which usually develops only after four to five hours.

2. It is impossible to separate the hypoglycemic effect from the diabetogenic action. Whenever hypoglycemia develops, diabetes follows if the animal survives.

Insulin assays in the pancreas of alloxan diabetic dogs. If the diabetes after treatment with alloxan is the result of the degeneration of beta cells, and the subsequent lack or decrease in insulin production, bio-assays of the pancreatic tissue should reveal a decreased insulin content.

Experiment VI. Assays were carried out on the pancreases of 3 dogs with alloxan diabetes of 18, 30 and 60 days' duration.[1] In all 3, the insulin content of the pancreatic tissue was markedly decreased (table 3). Whereas the pancreas of a normal dog contains about 2 to 3 units of insulin per gram, only $\frac{1}{4}$ of this amount was recovered from the alloxan treated organs. This seems to be conclusive evidence that

TABLE 4. COMPOUNDS TESTED FOR DIABETOGENIC ACTIVITY

1. UREIDES	2. OXIDISING SUBSTANCES
Alloxan	Sod. Nitro-Ferricyanide
Dialuric Acid	Sod. Molybdate
Alloxantin	Ceric Sulfate
Violuric Acid	Quinone
Barbituric Acid	Persulfate
N-Dodecyl Barbituric Acid	

3. QUINOLINE SUBSTANCES
Quinoline
Cinchophen

alloxan diabetes is a true pancreatic diabetes and is a result of insulin deficiency.

The specificity of the diabetogenic action of alloxan. Dunn and his coworkers (1943) had found that not only alloxan but also a quinoline compound (Styryl-Quinoline No. 90) produced islet cell necrosis in rabbits. They tested several other compounds as oxalic acid, uranium, guanidine and uric acid, and found that none of them had an effect on the islet cells or caused diabetes. Parabanic acid, uramil, violuric acid, benzol-barbituric acid, and thiouracil were examined by Thorogood (1944) and failed to produce comparable effects. Previously, Jacobs (1937) had found that none of a series of more than sixty chemical compounds, including most of the above mentioned substances, produced the fluctuation of the blood sugar level which is characteristic for the early response to alloxan.

Experiment VII. We have tested three groups of substances which are listed in table 4. The first group comprises compounds which are chemically related to alloxan, such as dialuric acid, the reduction product of alloxan, and alloxantin, which is formed by interaction of alloxan and dialuric acid. The second group represents various

[1] We are indebted to the Research Laboratories of Eli Lilly and Co., Indianapolis, where these bio-assays were carried out.

oxidising agents which were tested on the assumption that alloxan may owe its effect to its oxidising property. Quinoline and cinchophen were tested as relatives of styryl-quinoline. These substances in various doses were injected into dogs and the blood sugar level as well as the histological appearance of the pancreas were followed. None of them exhibited any diabetogenic activity though several proved to be very toxic.

The specificity of the diabetogenic action of alloxan therefore remains unexplained. It seems, however, unlikely that its oxidising property is the diabetogenic factor.

SUMMARY

Alloxan exerts its diabetogenic effect by a direct action upon the pancreas. The early hyperglycemia after alloxan is not a causal factor in the development of diabetes. Even if a normal blood sugar is maintained for an initial period after alloxan injection, islet cell degeneration and diabetes develop. The initial hyperglycemia depends on the presence of an intact adrenal medulla. Alloxan does not inhibit or inactivate insulin. The transitory alloxan hypoglycemia is produced by insulin liberated from degenerating pancreatic islet cells. In alloxan diabetes the insulin content of the pancreas is markedly decreased. The diabetogenic action is a specific property of alloxan. None of the chemically related compounds tested has a similar effect on carbohydrate metabolism.

Addendum: While this paper was in press, Ridout, Ham and Wrenshall published their data on the correlation of the insulin content and the histological picture of the pancreas in alloxan diabetes, and on the mechanism of alloxan hypoglycemia (*Science* 100: 57, July 21, 1944). Their findings are in good agreement with our experiments and conclusions.

REFERENCES

BAILEY, C. C., AND O. T. BAILEY: *J.A.M.A.* 122: 1165. 1943.

BRUNSCHWIG, A., J. G. ALLEN, M. G. GOLDNER AND G. GOMORI: *J.A.M.A.* 122: 966. 1943.

CAMPBELL, J., R. E. HAIST, A. W. HAM AND C. H. BEST: *Am. J. Physiol.* 129: 328. 1940.

CORKILL, A. B., P. FANTL AND J. F. NELSON: *M. J. Australia* 31: 285. 1944.

DUNN, J. S., H. L. SHEEHAN AND N. G. B. MCLETCHIE: *Lancet* 1: 484. 1943.

DUNN, J. S., J. KIRKPATRICK, N. G. B. MCLETCHIE AND S. V. TELFER: *J. Path. & Bact.* 55: 245. 1943.

DUNN, J. S., AND N. G. B. MCLETCHIE: *Lancet* 2: 384. 1943.

GOLDNER, M. G., AND G. GOMORI: *Endocrinology.* 33: 297. 1943.

GOLDNER, M. G., AND G. GOMORI: *Proc. Am. Diabetes A.* 1944, in press.

GOLDNER, M. G., AND G. GOMORI: *Proc. Soc. Exper. Biol. & Med.* 55: 73. 1944.

GOMORI, G., AND M. G. GOLDNER: *Proc. Soc. Exper. Biol. & Med.* 54: 287. 1943.

HARD, W. L., AND C. J. CARR: *Proc. Soc. Exper. Biol. & Med.* 55: 214. 1944.

HOUSSAY, B. A., V. G. FOGLIA, F. S. SMYTH, C. T. RIETTI AND A. B. HOUSSAY: *J. Exper. Med.* 75: 547. 1942.

HUGHES, H., L. L. WARE AND F. G. YOUNG: *Lancet* 1: 148. 1944.

JACOBS H. R.: *Proc. Soc. Exper. Biol. & Med.* 37: 407. 1937, personal communication.

LUKENS, F. D. W., F. C. DOHAN AND M. W. WOLCOTT: *Endocrinology* 32: 475. 1943.

THOROGOOD, E.: *Federation Proc.* 3: 48. 1944.

YOUNG, F. G.: *New England J. Med.* 221: 635. 1939.

THE EFFECTS OF PITRESSIN TANNATE AND WATER RESTRICTION ON THE WATER EXCHANGE AND RENAL FUNCTION OF NORMAL DOGS

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A SUSPENSION of pitressin tannate in oil[1] (PTO) has proved effective in controlling the polydipsia and polyuria of experimental and clinical diabetes insipidus (Blotner, 1942; Court & Taylor, 1943; Greene & January, 1940 & Thorn & Stein, 1941). The effect of a single intramuscular injection persists for a period of 24 to 96 hours in contrast to the evanescent action (3 to 6 hours) of the aqueous preparation (Blotner, 1942). The prolonged action of pitressin tannate is probably due primarily to its slow absorption from the site of injection. As far as we are aware, there has been but one report dealing with the activity of successive injections of pitressin tannate on normal animals, rabbits (Dexter and Weiss, 1941). This communication presents some studies on the effect of PTO on the water exchange and renal concentrating power of normal dogs and in addition, its effects are compared with those produced by a prolonged period of water limitation.

METHODS

The experiments were performed on 1 male and 8 female dogs weighing from 8 to 16 kg. The animals were kept under observation for 2 to 14 months in individual metabolism cages and were fed a meat diet (20 to 30 gm. per kg.) to which were added yeast, cod liver oil, bone ash, and 2 gm. of sodium chloride daily. The female animals, previously prepared by episiotomy, were catherized at the end of each 24 hour period, using a 10F rubber catheter. The male dog was catheterized every 72 hours by means of an 8F woven silk catheter. The body weight, the water intake, and the volume, specific gravity, and chloride content of the urine were determined daily. At intervals, venous blood was drawn from a leg vein without stasis or anesthesia. Hemoglobin was determined by the acid hematin method using a photoelectric colorimeter (Klett-Summerson); the hematocrit on heparinized blood in Sanford-Magath tubes; plasma specific gravity by the method of Barbout

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[1] Generously furnished by Parke Davis and Company.

and Hamilton; plasma chloride (Eisenman, 1929; Campbell and Hanna, 1937); NPN (Drew, Scudler and Papps, 1940); and serum CO_2 (van Slyke and Neill, 1924) content by routine laboratory methods. The urine specific gravity was determined by means of the Westphal balance.

After a control period during which basal data were obtained, the dogs received 5 pressor units of pitressin tannate in 1 cc. of oil subcutaneously each morning. Before, during and following the pitressin period, the diuretic response to a water load of 25 cc. per kg. (Pasqualini, and Avogadro, 1942; Stehle and Bourne, 1925) was measured over a 3 hour period. Several weeks after a series of pitressin injections the water intake of some of the animals was restricted to the average amount taken voluntarily while under the influence of pitressin tannate.

RESULTS

Table 1 shows the effects produced by the daily administration of 5 units of PTO. In 1 male and 7 female dogs there was a rise in the average urine specific gravity, a decrease in the average water intake of 8 to 40 per cent, and a fall in the average urine volume of 8 to 30 per cent. In dog 3, a second series of injections performed during a hot spell was attended by an increase in water intake of 20 per cent, but there was a decrease in urine volume of 23 per cent and a rise in urine specific gravity. In dog 8 the fluid balance and urine specific gravity were unaffected by 12 daily injections.

TABLE 1. THE EFFECTS OF PITRESSIN TANNATE AND WATER RESTRICTION ON WATER EXCHANGE AND RENAL CONCENTRATING POWER

Dog no.	Control Average of last 7 days					Pitressin tannate in oil 5 U. in 1 cc. daily					
	Water intake cc.	Urine output cc.	Urine sp. gr.	Urine chloride m. eq.	Plasma chloride m. eq.	No. injections	Water intake cc.	Urine output cc.	Urine sp. gr.	Urine chloride m. eq.	Plasma chloride m. eq.
1	152	179	1.044	35.0	121	21	100	125	1.057	36.0	120
2M	215	184	1.043	29.0	103	12	139	149	1.054	31.0	113
3	200	185	1.043	42.0	114	8	241	142	1.053	35.0	119
3	200	170	1.042	35.0	111	10	175	131	1.053	34.2	115
4	363	310	1.023	33.0	108	8	333	231	1.031	36.9	114
5	230	224	1.042	34.2	113	7	208	206	1.049	36.4	106
6	299	223	1.032	33.4	107	7	261	178	1.043	33.4	110
7	182	173	1.049	35.0	118	7	118	144	1.062	37.0	121
8	117	157	1.051	38.0	118	12	128	157	1.052	36.0	120
9	183	240	1.039	36.0	123	11	140	210	1.048	38.0	125
Average	204.1	204.5	1.0408	35.0	114		184.3	167.3	1.0502	35.4	116

Dog no.	Water limitation						Per cent of water load as urine	
	No. of days	Water intake cc.	Urine output cc.	Urine sp. gr.	Urine chloride m. eq.	Plasma chloride m. eq.	During control period	During pitressin period
1	9	100	126	1.054	36.0	120	52	5.0
2M	10	140	131	1.057	30.0	113	72	3.5
3							75	7.0
3	10	185	150	1.052	38.0		75	4.0
4	10	330	167	1.047	37.6		63	6.0
5	7	208	218	1.043	38.8	109	75	5.0
6	8	260	193	1.036	35.0	117	75	5.0
7	7	120	130	1.065	35.0	117	74	11.0
8	10	125	164	1.043	32.2	117	46	5.0
9	11	140	175	1.046	34.7	116	75	5.0
Average		170.8	157.9	1.0497	35.3	116	68.2	5.6

The first day responses to the injection of PTO on 8 female dogs are presented in Table 2. In 6 dogs there was no change in urinary output, in spite of the fact that 3 of these showed an increase in the water intake. In 5 of the dogs there was an increase in the urinary excretion of chloride of from 10 to 21 m. eq.

During the following days of pitressin administration (Table I) the urine volume fell from 204 cc. to 167 cc. while the specific gravity remained high. The initial loss of chloride from the 5 dogs was apparently compensated for by a subsequent retention since there was no significant increase in the average daily excretion of chloride in the urine during the PTO period in any of the dogs. In dog 3 whose

TABLE 2. CHANGES PRODUCED BY A SINGLE INJECTION OF PITRESSIN TANNATE

Dog no.	Excretion on day before pitressin				24 hrs. after pitressin injection			
	Water intake cc.	Urine volume cc.	Urine sp. gr.	Urine chloride m. eq.	Water intake cc.	Urine volume cc.	Urine sp. gr.	Urine chloride m. eq.
1	140	140	1.049	30.0	80	169	1.048	43.1
3	180	145	1.045	31.5	100	150	1.050	41.3
4	325	260	1.023	27.0	390	275	1.026	48.0
5	250	230	1.044	23.7	310	178	1.048	24.1
6	210	185	1.034	33.8	240	195	1.041	35.8
7	200	160	1.052	33.5	100	154	1.063	44.1
8	135	160	1.052	34.6	170	162	1.052	32.7
9	170	226	1.043	31.1	175	231	1.049	41.5
Average	201	188	1.0427	30.65	196	189	1.0471	38.83

chloride excretion was excessive during the control period, the average daily chloride output fell during the administration of PTO.

During the PTO period there was no significant alterations in the hemoglobin concentration, in the hematocrit value, the non-protein nitrogen concentration, or the plasma specific gravity. In 5 of the dogs, there was noted moderate increases in plasma chloride concentration of from 2 to 10 m. eq. The dogs showed no "ill" effects. There was no evidence of anorexia such as was noted by (Dexter and Weiss, 1941) in rabbits receiving repeated injections of pitressin tannate. There was also no evidence of any condition resembling adrenal insufficiency as reported by (Britton and Klein, 1942) in dogs. The only sign of excessive stimulation of some of the smooth musculature was that occasionally the stools were moist during the first days of the pitressin period.

From the study of the daily water balance alone there may be little or no evidence that a dog receiving pitressin tannate is under its influence (Stehle and Bourne, 1925). This phenomenon is due to the fact that the normal dog drinks no excess of water and normally concentrates its urine very nearly maximally. However, evidence of pitressin anti-diuretic activity can be obtained readily by observing the rate of urine formation when water is administered to the dog. Following the intragastric administration of 25 cc. of water per kg. the dogs were catheterized at 30 minute intervals for 3 hours. In

every one of the 9 dogs there was complete absence of the diuresis which occurs normally, that is, in the absence of pitressin (Table 1). This amount of water was not followed by any obvious symptoms of water intoxication. In the 24 hours following the water load experiment the dogs did not drink any water voluntarily, while occasionally no water was taken for as long as 48 hours.

When the daily injections of PTO were discontinued the water exchange and the urine specific gravity returned to pre-injection levels. These levels were attained at approximately 2 to 3 days in 7 of the dogs which had received the hormone for 1 to 12 days, while in the dog which received injections for 21 days, normal levels were reached after 7 days. In dogs 1 and 9, which were tested with a water load of 25 cc. per kilo by stomach tube 5 and 9 days after the cessation of injections of pitressin tannate, there was still present a maximal inhibition of water diuresis despite the fact that the daily fluid balance had returned to its pre-injection level. These facts indicate that in the normal dog changes in the daily fluid exchange cannot be used as criteria of pitressin anti-diuretic activity.

Several weeks following the completion of the PTO periods, the dogs in whom the water intake and urine volume had been decreased by the hormone were restricted to a water intake equal to the average daily amount of water which had been taken voluntarily while under the influence of PTO. It is apparent from the results, which are shown in Table I for easy comparison with the effects of pitressin, that there was only one significant difference between the effects of PTO and experimental moderate water limitation. The difference in the reaction of the animals in the 2 experiments was the development of intense thirst during the water limitation in contrast to the disregard for water shown by the dogs receiving PTO. In patients with diabetes insipidus who had been deprived of water for many hours, pitressin produced alleviation of the sensation of thirst in 20 minutes (Pasqualini and Avogadro, 1942). The reason for this difference cannot be ascertained from our data, but it is probably related to the effects of PTO upon the electrolyte balance in the normal dog (Stehle and Bourne, 1925) and possibly upon the central receptors of thirst (Pasqualini and Avogadro, 1942). At the termination of the periods of water limitation the dogs drank 400 to 800 cc. of water immediately and retained most of it as indicated by low levels of urine flow and the high urine specific gravity during the 3 hour period following the drinking of the water.

DISCUSSION

A moderate oligodipsia and oliguria with urine of high specific gravity were the characteristic responses of normal dogs to a series of injections of PTO. There were no significant changes in the average daily urinary excretion of chloride or in the composition of the plasma as measured by hematocrit values or the plasma chloride and specific

gravity. These findings are in contrast to those reported by (Britton and Klein, 1942) who used large doses of aqueous post-pituitary extracts.

A temporary increase in the excretion of chloride in the urine occurred in 5 of the 8 dogs following an injection of pitressin tannate in oil. This phenomenon is probably analagous to the initial increase in urinary chloride which follows a single dose of aqueous pitressin and which is often associated with an increase in the volume of urine (Mulinos, Spingarn and Lojkin, 1941; Thorn and Stein, 1941). It seems possible that these initial changes may be due to small amounts of the oxytocic hormone present in the PTO used (Fraser, 1942). Despite continued administration of PTO the increase in the excretion of chloride was maintained only during the first 24 hours so that it is probably an extraneous action of pitressin and not part of, but incidental to the anti-diuretic activity, such as that suggested by (Fraser, 1942).

The first day effects of PTO on water balance and chloride excretion are the reverse of those which occur following daily injections of desoxycorticosterone acetate (DCA) (Mulinos, Spingarn and Lojkin, 1941). Dogs injected with DCA retain chloride and water during the first 24 hours and before there is any evidence of the polyuria which follows. It was suggested previously (Mulinos, Spingarn and Lojkin, 1941; Smith, 1937) that pitressin and DCA are antagonistic in their effects upon the daily water balance. The facts adduced in the present report support this hypothesis and lend further credence to the concept that among other factors the normal water balance involves the physiological activity of both of these substances.

Our results obtained on 9 dogs iterate the fact that oligodipsia and oliguria with urine of high specific gravity are not reliable criteria of pitressin anti-diuretic activity. Indeed, in dogs apparently unaffected by pitressin, a water load given by stomach tube is not followed by any increased diuresis, giving ample evidence of anti-diuretic activity.

A series of daily PTO injections and a period of daily water limitation produced similar changes in the volume and specific gravity of the urine of normal dogs. This demonstration that the normal kidney tubules reabsorb water equally well during thirst and following supramaximal amounts of pitressin is in accord with the concept that the posterior lobe principle of the pituitary enables the kidney to reabsorb water maximally in response to the need for water conservation.

SUMMARY

Eight normal dogs received daily injections of 5 units (1 cc.) of PTO. The urine volume decreased 8 to 30 per cent while its specific gravity rose and water intake decreased 8 to 40 per cent. There was

no alteration in hemoglobin, hematocrit or NPN values or in plasma specific gravity. After the first day the urine chloride excretion did not change during the injections. On the first day, 5 of the dogs showed no change in urine volume and an increase in the excretion of chloride of 10 to 21 m. eq. A water load of 25 cc. per kilo given by stomach tube was retained maximally. The dogs refused water for 24 to 48 hours after such a test. After the PTO injections were discontinued, the fluid balance returned to normal in 2 to 7 days depending on the number of injections (7 to 21). However, as long as 9 days after discontinuance of PTO a water load given as above was completely retained. When the dogs have recovered completely from the effects of PTO and are then limited to a water intake equal to that taken voluntarily during the injections period, the blood and urinary findings are analogous to those under PTO, but the dogs become extremely thirsty.

REFERENCES

BLOTNER, H.: *J.A.M.A.* 119: 995. 1942.
BRITTON, S. W., AND R. F. KLEIN: *Federation Proc.* 1: 10. 1942.
CAMPBELL, W. R., AND M. HANNA: *J. Biol. Chem.* 119: 1. 1937.
COURT, D., AND S. TAYLOR: *Lancet.* 1: 265. 1943.
DEXTER, L., AND S. WEISS: *Preeclamptic and Eclamptic Toxemia of Pregnancy:* Boston, Little Brown & Co. p. 80. 1941.
DREW, C. R., J. SCUDDER AND J. PAPPS: *Surg., Gynec. & Obst.* 70: 859. 1940.
FRASER, A. M.: *J. Physiol.* 101: 236. 1942.
EISENMAN, A. J.: *J. Biol. Chem.* 82: 411. 1929.
GREENE, J. S., AND L. E. JANUARY: *Proc. Soc. Exper. Biol. & Med.* 44: 217. 1940.
MULINOS, M. G., C. L. SPINGARN AND M. E. LOJKIN: *Am. J. Physiol.* 135: 102. 1941.
PASQUALINI, R. Q., AND A. C. AVOGADRO: *Rev. Soc. Argent. de biol.* 18: 363. 1942.
SMITH, H.: *Physiology of Kidney.* Oxford Univ. Press, New York. p. 210–234. 1937.
STEHLE, R. L., AND W. BOURNE: *J. Physiol.* 60: 229. 1925.
STEPHENS, K. V.: *Proc. Soc. Exper. Biol. & Med.* 44: 240. 1940.
THORN, G. W., AND K. E. STEIN: *J. Clin. Endocrinol.* 1: 680. 1941.
UNNA, K., AND L. WATTERSKIRCHEN: *Arch. f. exper. Path. u. Pharmakol.* 181: 681. 1936.
VAN SLYKE, D., AND J. M. NEILL: *J. Biol. Chem.* 61: 523. 1924.

ON BROODINESS OF RING DOVES FOLLOWING IMPLANTS OF CERTAIN STEROID HORMONES

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THE INDUCTION of broodiness in laying fowl by injections of prolactin was reported by Riddle, Bates and Lahr (1935a). The same observers (1935b, 1942) later found that in intact and operated rats of both sexes maternal behavior may result, directly or indirectly, from injections of certain pituitary and steroid hormones. The literature on this subject was reviewed in the publication of 1942. The purpose of the present study was to learn whether those steroids which had proved effective in rats were likewise active in birds, and simultaneously to learn whether the effective steroid hormones do or do not give evidence of increasing the output of prolactin from the pituitary of the treated dove. Only doves or pigeons can be used for this last-named test since other birds cannot similarly indicate an increased output of prolactin through enlargement of crop-sacs. Preliminary tests reported by Riddle and Schooley (1938) indicated that the daily handling and injection of doves often disturbed or terminated broodiness even when this behavior had become well established in the normal manner; also that implantation of pellets did not produce such disturbance and, in the case of progesterone, implantations were followed by broodiness in male doves.

PROCEDURE

It is probable that none of the 133 birds used in these tests had previously experienced broodiness; the 25 adult females (18 tests; 7 controls) had each laid one or two eggs, but had been stopped short of actual exhibition of broodiness by transfer of the bird to another cage just before laying and by immediate removal of the egg and nest at the time of laying. The dove attains adult size when 10 to 12 weeks old and becomes sexually mature at 6 to 10 months. The birds listed as young were 3 to 4 months old; those called adolescent were 4 to 6 months old; the adult birds were known to be sexually mature and were 6 to 10 months old.

The pellets were implanted subdermally over the breast with a blunt (No. 7) trocar. Two birds of the same sex and approximate age were given similar implants on the same day; thereafter this pair was kept together in

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a large (2 cubic meters) well-lighted breeding cage in the *front* of which, nearest to the passageway and the observer and at a height of 4 or 5 feet from the floor, an adequate nest was provided. On the following day a "dummy" dove egg was placed in the nest. During this day, and 3 or 4 times daily thereafter for the duration of the test, the behavior and location (whether on perches, on floor, on or *in* nest-box) of the two birds were recorded. The control birds received no implants but were otherwise treated and observed in the same manner. The pellets were removed after 8 to 14 days, though observations extended over a period of three weeks and for a longer period when ability of broody birds to rear young was tested. A limit of three weeks of observation was also imposed in tests on control birds since some published (Whitman, 1919) and much other personal experience had shown that more prolonged periods may result in the pairing (and later in broody behavior) of two doves of the same sex. Several pairs of birds were given fertile eggs after they became continuously broody; or, after brooding for 14 days, a newly hatched dove was substituted for the "dummy" egg. In this way 18 tests were made, with positive results in all cases, of the ability to rear young subsequent to the broodiness induced by the three effective steroids.

Only birds which have been broody for at least 14 days may be expected to have fully developed crop-sacs and be capable of "secreting" crop-milk for a newly hatched squab. Some of the birds which were feeding young, and several other birds whether broody or not were killed to obtain the weights of their crop-sacs. Some birds which gave the essential test—namely, of "nesting" on three successive days and permitting the observer's hand to be placed repeatedly in the nest beneath their breasts—were otherwise very irregular in their broody behavior. In a few cases responding birds ("reactors") later lost all interest in the nest and were not broody during an entire cycle. A few tests were made with single birds completely isolated from any other bird; earlier personal experience had shown that this condition usually opposes the egg-production which normally precedes broodiness in female doves.

Birds treated with progesterone were somewhat sluggish and soon developed intestinal stasis. Pellets of 1.5 to 3.0 mg. of this steroid therefore proved more satisfactory than larger ones; in most cases pellets of progesterone were removed at the end of 8 days. The larger pellets (6 to 12 mg.) of other steroids were all well tolerated. None of the steroids administered by this method resulted in loss of body weight, and pellets of desoxycorticosterone acetate (DCA) regularly increased the body weight.

RESULTS

The results are shown in table 1. Pellets of progesterone, DCA and testosterone propionate induced broodiness in many or most tests except in the case of isolated (unpaired) birds and the very young. Broodiness did not occur in any of the 39 controls nor in birds treated with either estrone (10 tests) or 11-dehydrocorticosterone (4 tests). The latter steroid was not tested adequately. Adult birds of either sex were usually more responsive than younger birds. Most of the responding birds ("reactors") became broody promptly at 3 to 7

days after implantation, and 38 of the total of 45 positive responses were obtained within 9 days of the date of implantation. Likewise, when active sex behavior was present in males it was completely suppressed within 1 to 3 days after receiving pellets of progesterone, estrone or DCA. Some female doves treated with testosterone propionate cooed in male fashion and, like a male, sometimes drove their

TABLE 1. THE ABILITY OF CERTAIN STEROID HORMONES (PELLET IMPLANTATIONS) TO INDUCE BROODY BEHAVIOR IN RING DOVES

Birds used		Percentage of reactors		Crop-sac weights of				Number that reared young
Age group and sex	Number	Brooded eggs	Fed young	Reactors	No.	Non-reactors	No.	
				mg.		*mg.*		
Progesterone								
Adult ♂	12	100	80*	2110	4			6
Adult ♀	6	100	100	(4560)	2			2
Isolated ad. ♀	3	0	0			422	3	0
Adolescent ♂	6	33	33			425	2	2
Adolescent ♀	4	25	0†	400†	1	373	3	0
Young ♂	4	0	0			390	1	0
Young ♀	5	20	no test	690	1			0
Desoxycorticosterone acetate								
Adult ♂	6	100	33	1766	4			2
Isolated ad. ♂	6	17	no test	2670	1			0
Adult ♀	4	100	100	4620	2			2
Adolescent ♂	6	50	33	2605	3	380	1	0
Adolescent ♀	4	25	25	3310	1	310	1	0
11-dehydrocorticosterone								
Adolescent ♂	4	0	0			375	4	0
Testosterone propionate								
Adult ♀	8	50	(?)‡	2250	2	388	4	2
Adolescent ♀	6	66	33	1750	2	405	1	2
Estrone								
Adult ♂	4	0	0			405	1	0
Adolescent ♂	6	0	0			370	4	0
Control								
Adult ♂	8	0	0			290–440	5	
Adult ♀	7	0	0			302–370	5	
Adolescent ♂	14	0	0			320–430	6	
Adolescent ♀	6	0	0			390	1	
Young ♂	4	0	0			360	1	

* On the basis of 10 tests; 2 birds not tested.
† A single bird became broody but died only 7 days later.
‡ Only 2 of the 4 broody birds were tested here; both gave food to nestling young.

associate about the cage. Occasionally this behavior persisted for several days after removal of the pellet of testosterone.

Crop-sacs were enlarged in birds treated with steroids which induced broody behavior; but, in the restricted number of cases thus examined, this occurred only in those individuals which actually became broody under the treatment. The 29 birds which fed or reared young also proved by this performance that their crop-sacs had been

stimulated (enlarged) though such birds were usually not killed to obtain weights of their crop-sacs. Crop-sacs were not enlarged by treatment with steroids which did not induce broody behavior, nor in any of 18 control doves on which this measurement was made.

DISCUSSION

It was earlier shown (Riddle, Bates and Lahr, 1935a) that prolactin induced broodiness in suitable fowl while gonadotrophin and thyrotrophin were ineffective. The three steroid hormones which were found to have some or much ability to release broody behavior in the present tests showed also concurrent ability to enlarge the crop-sac of these doves; it is practically certain that the latter effect is produced by prolactin only. Though this group of hormones can be effective in producing these two responses the considerable body of evidence obtained indicates that broodiness did not occur unless prolactin was released. Unless the enlargement of the crop-sacs was initiated the doves did not become broody. Again, estrone showed no ability to enlarge crop-sacs and failed also to induce broodiness. It is probable, therefore, that the steroids here found capable of releasing broody behavior accomplish this indirectly by causing a release of prolactin from the dove's own pituitary. The data obtained on weights of crop-sacs, and the ability of the responding birds to feed young after 14 days of broodiness induced by the implants, indicate that the pituitaries of these birds, like the normal brooding birds studied by Lahr and Riddle (1938), were supplying increased amounts of prolactin from the very beginning of broodiness. The unstimulated condition of the crop-sacs of the non-reactors is evidence that such birds were not receiving this same stimulation. Moreover, since the pellets were usually removed during the first week of incubation with little or no interruption of broodiness (when this had been established) and with several proved cases of continued enlargement of the crop-sacs during the following week, it also seems certain that these cases of continued broodiness were associated with continued production of prolactin.

Part of the wide difference in weight between the crop-sacs of different "reactors" resulted from making the measurement at unequal periods after the initiation of broodiness. Another part of these variations—a few crop-sacs enlarged less than in other broody birds—seems to be associated with defective or interrupted broodiness in these particular doves. Burrows and Byerly (1936) reported that pituitaries taken from broody hens contain more prolactin than do those from laying hens. Laqueur and Fluhmann (1942) concluded that testosterone propionate inhibits the output of LH and increases the output of prolactin and FSH by the rat pituitary. Reece and Bivans (1942) observed that the prolactin content of pituitaries of spayed rats is increased by injections of progesterone.

The present data relate to parts only of the mechanisms involved in the hormonal control of broodiness and parental care; but specific steroids seem capable of increasing the output of prolactin by the bird's pituitary and when this occurs broodiness also ensues. If this same relationship between steroids and pituitary output of prolactin holds in rats it is probable that there also prolactin is more *directly* related to the induction of maternal behavior (Riddle, Lahr and Bates, 1942) than are the effective steroids. The reason for some failures of doves to react in the present tests is not clear although the degree of maturity of the bird is one factor and variations in the amount of hormone absorbed may be another. Perhaps a positive response may be favored and augmented by the antigonad action of the effective steroids, and even by the antigonad action of prolactin whose output is increased when positive responses are obtained. Clearly, however, antigonad action is not solely involved, since estrone is ineffective in producing broodiness though it is known to be strongly antigonad in the male dove (Bates, Riddle and Lahr, 1937; Lahr and Riddle, 1944). All of the steroids which produced broodiness in these tests—progesterone, testosterone propionate and DCA—were earlier found effective in producing maternal behavior in rats (Riddle, Lahr and Bates, 1942). Estrone was not effective in rats, and in 10 tests on male doves it neither induced broodiness nor released prolactin from their pituitaries.

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SUMMARY

Ring doves of both sexes and of three age levels were used to test the ability of implanted pellets of certain steroid hormones to induce broodiness and to release prolactin from the pituitaries of the treated birds. Seventeen untreated pairs of mature or adolescent doves served as controls and these, together with 5 pairs treated with pellets of estrone (total of 44 birds), did not become broody nor did their crop-sacs increase in size. Paired adult and adolescent birds of either sex treated with progesterone or desoxycorticosterone acetate, and similar females treated with testosterone propionate, became broody in 43 of 62 tests. Twenty-one of these broody birds were killed while broody, or while feeding young, and proof of enlargement of their crop-sacs was obtained by weighing these organs; a total of 29 of these responding birds fed or reared young, thus proving both the functional state of their crop-sacs and the antecedent release of prolactin under the stimulus of the effective steroids. These steroids were nearly or quite ineffective in young doves and in isolated (unpaired) mature doves. Those steroid hormones which were earlier observed by Riddle, Lahr and Bates to promote maternal behavior in rats were here shown to

be effective in inducing broodiness in doves. This effect in doves was produced by steroids which caused a concurrent release of prolactin from the dove's own pituitary but not by steroids which failed to do so. This result provides further evidence for a specific rôle of prolactin in the initiation of broodiness and parental care.

REFERENCES

BATES, R. W., O. RIDDLE AND E. L. LAHR: *Am. J. Physiol.* 119: 610. 1937.

BURROWS, W. H., AND T. C. BYERLY: *Proc. Soc. Exper. Biol. & Med.* 34: 841. 1936.

LAHR, E. L., AND O. RIDDLE: *Am. J. Physiol.* 123: 614. 1938.

LAHR, E. L., AND O. RIDDLE: (In press).

LAQUEUR, G. L., and C. F. FLUHMANN: *Endocrinology* 31: 300. 1942.

REECE, R. P., AND J. A. BIVANS: *Proc. Soc. Exper. Biol. & Med.* 49: 582. 1942.

RIDDLE, O., R. W. BATES AND E. L. LAHR: *Am. J. Physiol.* 111: 352. 1935a.

RIDDLE, O., E. L. LAHR AND R. W. BATES: *Proc. Soc. Exper. Biol. & Med.* 32: 730. 1935b.

RIDDLE, O., E. L. LAHR AND R. W. BATES: *Am. J. Physiol.* 137: 299. 1942.

RIDDLE, O., AND J. P. SCHOOLEY: *Carnegie Inst. of Wash., Year Book.* 37: 56. 1938.

WHITMAN, C. O.: The Behavior of Pigeons. *Carnegie Inst. of Wash. Publ. No. 257*, 3: 1919.

THE ACTION OF STEROID HORMONES ON THE MATURE DOVE TESTIS

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THE MODE OF action of androgens on the mature testis of both birds and mammals is still an unsolved problem. For both classes of vertebrates there are data which indicate that androgens sustain or stimulate the testis, and other data which indicate that in mature testes these hormones produce one or another degree of testicular atrophy. Some of these discordant results are perhaps ascribable to the use of different levels of dosage; the use of impure steroid preparations in some earlier investigations probably led to erroneous results. The effect of estrogens on the mature testis is less questionable, but data for the bird testis are few and inadequate. The action of crystalline progesterone, stilbestrol and desoxycorticosterone acetate on the testes of mature birds has not hitherto been reported.

Morató-Manaro, Albrieux and Buño (1935) injected each of several cocks with estrone in doses up to 50,000 I.U. during about one week and observed that their testes were smaller than normal, that they were white, and that there was a reduction of both spermatogenic and interstitial tissue. These authors (1938) later reported that inunction of estrogen for 10 days in White Leghorn cockerels of 3 months caused atrophy of the testes; also that in such birds the inunction of androsterone (probably Erugon) was still more effective than estrogen in causing testicular atrophy. Bates, Riddle and Lahr (1937) reported that in small groups of adult male doves injected for 10 days with non-crystalline preparations of progesterone, androsterone and estrone the testes lost approximately 80 per cent of their pretreatment weight. Zondek (1937) gave huge doses of estradiol benzoate to fowls from the age of six weeks to the time the control birds were mature. The injected "pituitary dwarfs" were only half as large as the control and their testes were only 3 to 4 per cent of the normal weight. Breneman (1937) reported that testosterone and dehydroandrosterone retarded testis growth in very young chicks but accelerated testis growth in chicks 30 days old. Pfeiffer and Gardner (1938) treated adult male pigeons with 1000 I.U. of estrone daily for 22 days and observed a reduction of the testes to one-tenth of their original

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weight. These authors further found that when as much as 1.25 to 2.5 mg. daily of testosterone propionate was added to the estrone the weight of the testes was unaffected. Emmens (1939) gave heavy doses of estrone or estradiol to 5 Leghorn cocks which survived treatment for various long periods and observed very marked atrophy of the testes in all cases. Chu (1940) reported a loss of testis weight in 3 normal pigeons injected for 30 days with 2 mg. daily of testosterone; but in 3 hypophysectomized pigeons, similarly treated and observed at laporotomy (19 days), testis weight was partly supported. The literature dealing with the action of androgens on the mammalian testis was recently reviewed by Moore and Morgan (1942).

Other studies conducted in this laboratory made it desirable to know the effect of still other steroids—particularly progesterone and desoxycorticosterone acetate (DCA)—on the mature dove testis. Both of these steroids have been found capable of inducing broodiness in ring doves (Riddle and Lahr, 1944) and maternal behavior in rats (Riddle, Lahr and Bates, 1942); prolactin and phenol were also effective in rats. In all of these cases it is important to learn whether those substances which induce broodiness (or maternal behavior) do or do not have an "anti-gonad" action. In the present tests progesterone and phenol were observed to have some ability, while desoxycorticosterone acetate and prolactin showed very great ability to induce testicular atrophy in doves.

MATERIALS

Tests were made on three series of adult male ring doves; 81 experimental and 22 injected control doves were used. Since the weight of testes of adult doves varies both individually and seasonally a biopsy was performed and weight of the testes was estimated. These estimates involved a possible error of less than 20 per cent. Only birds with apparently healthy testes of medium or large size were accepted for the test. The accepted birds were so adjusted into groups of 4 to 8 that the several groups had testes of approximately the same total weight at the time treatment was started. One of these groups served as control and was injected with sesame oil only. Injections were started one or two days after biopsy, and body weights were recorded at the time of first injection and at the end of the test. Since fasting (voluntary or otherwise), wholly apart from any other treatment, produces rapid atrophy in the testes of doves and pigeons it is necessary to observe changes in body weights in order that voluntary fasting could be detected or excluded. None of the birds treated in the present study lost the amount of weight which would accompany a marked decrease of food consumption (see table 1).

Each group was maintained in a separate pen where all birds had free access to a surplus of food and water. At the end of 10 or 14 days of daily injections the birds were killed and the final weight of their testes obtained. In certain tests a pellet weighing 8 to 12 mg. was implanted subdermally into the neck in addition to the daily injections of the hormone. The steroids[1]

[1] All of the steroids used in this study were crystalline products contributed by the Schering Corporation through the courtesy of Dr. Erwin Schwenk. Diethylstilbestrol was contributed by Sharpe and Dohme.

injected were previously dissolved in sesame oil. Prolactin and phenol were in aqueous solution.

RESULTS

Effects on testis weight. The data of table 1 show a pronounced atrophy of the testes following injections of estrone, estradiol benzoate, stilbestrol, desoxycorticosterone acetate and prolactin. Progesterone exerted a definite, but less pronounced, depressant action on the testes along with a very marked increase of body weight. Its effect upon the testes, however, was much less than that earlier ob-

TABLE 1. THE ACTION OF VARIOUS STEROID HORMONES AND CERTAIN OTHER SUBSTANCES ON THE TESTES OF ADULT DOVES. INJECTIONS MADE DAILY OVER 10 DAYS

Treatment	Dosage daily	Number	Change in body weight	Testes	
				Final weight	Per cent of control
			gm.	*mg.*	
Sesame oil, control	0.2 cc.	8	+ 6	1204	(100)
Physiological saline	0.4 cc.	5	+ 8	1294	(100)
Testosterone	2.0 mg.	4	+ 1	1217	101
Testost. prop. + pellet	2.0 mg.	8	+ 9	1061	88
Cholestenone + pellet	3.0 mg.	4	+ 7	1050	87
Progesterone + pellet	2.0 mg.	4	+25	881	73
Phenol—0.5% solution	0.2 cc.	4	+ 6	996	83
Androsterone + pellet	2.0 mg.	8	+12	1469	123
Desoxycort. ac. + pellet	2.0 mg.	8	+17	172	14
Prolactin, 706 (50 I.U.)	5.3 mg.	4	+20	275	22
Sesame oil, control	0.2 cc.	5	+ 3	1195	(100)
Estradiol benzoate	0.5 mg.	5	+ 4	179	15
Stilbestrol	0.5 mg.	4	+ 2	177	15
Androsterone	0.8 mg.	4	+ 8	1505	126
Sesame oil, control	0.2 cc.	4	+ 7	755	(100)
Estrone	0.5 mg.	4	− 3	130	17
Desoxycort. ac. + pellet	2.0 mg.	4	+14	156	21
Androsterone + pellet	2.0 mg.	3	+ 3	1053	139
Testosterone	4.0 mg.*	4	+ 0	799*	106
Testosterone	0.8 mg.*	4	+ 8	616*	81

* The period of injection was 14 days.

served (Bates, Riddle and Lahr, 1937) following the injection of another preparation (Proluton). When administered at a level of 2 mg. or 4 mg. daily testosterone had no significant effect on testis weight. The lowest dosage used (0.8 mg. per day) was apparently associated with a slight loss of testis weight, but the test was not repeated and its significance is questionable. The testes of three groups (15 birds) treated with androsterone increased to 123, 126 and 139 per cent of their weight prior to injection.

Cholestenone probably produced no effect; others have noted that this steroid does not possess hormonal activity. Phenol probably caused a slight loss of testis weight; the observed decrease is perhaps not significant but the histological data showed definite damage to the Leydig cells.

Slight decreases of testis weight observed under dosage with phenol and with 0.8 mg. testosterone may represent atrophy of the interstitial cells alone. Selye and Friedman (1941) observed interstitial cell atrophy in the rat following injections either of steroids in general or of other non-specific agents.

Histology.—The testes of one or more typical birds from each group were preserved and examined histologically. When the loss of testis weight was less than 30 per cent there was usually only slight histological evidence of damage to germinal tissue; this evidence consisted in an apparent decrease in size of the individual tubules and in a reduction in the number of mature sperm. The much greater losses in testis weight following injections of the estrogens, prolactin and desoxycorticosterone acetate were accompanied by very marked histological changes. Tubules were then greatly reduced in size, very few or no sperm were present, lumens were filled with cellular debris, and a high proportion of the germinal cells had pyknotic nuclei.

Only small amounts of interstitial tissue (Leydig cells) are present in the normal adult testis of the dove, and the influence of damaging agents on this tissue is not easily estimated when total testis weight is greatly changed. Indeed, even apart from changes in total testis weight, changes in Leydig cells are not easily evaluated. Shrunken cells and reduction in number of cells were used as the main criteria of atrophy of Leydig cells. Testosterone and its propionate, at levels of 0.8 mg. and 2.0 mg. daily, produced atrophy of the Leydig cells. Atrophy resulted also from injections of desoxycorticosterone acetate, phenol and estrone. Progesterone probably caused slight damage. The condition of the interstitial cells after injections of androsterone was somewhat obscured by the increase of tubular tissue; no damage was noted. The prolactin used in these tests did not damage Leydig cells. This result is perhaps to be expected since the prolactin preparation used in these particular tests slightly enlarged the seminal vesicles of 21-day rats. The loss of 78 per cent of testis tissue under dosage with prolactin appears wholly due to atrophy of germinal elements. It was previously reported (Lahr, Riddle and Bates, 1935) that prolactin preparations which cause atrophy of all elements of the dove testis resulted, after injection for 13 days, in a loss of approximately 92 per cent of the gonad weight.

DISCUSSION

Selye and Friedman (1941) showed that suitable dosage levels of testosterone, estrone and desoxycorticosterone acetate, like non-specific agents such as formaldehyde, all lead to atrophy of Leydig cells and of the tubular epithelium in the adult testis of the rat. In that study progesterone caused only a slight atrophy of the interstitial cells. Selye and Albert (1942) later concluded that all hormonally active steroids injure the Leydig cells, and that steroids with

greatest estrogenic (folliculoid) potency produce the most pronounced atrophy of Leydig cells. Nelson (1936) had earlier found that androgens and progestin are similarly effective in their support of testicular tubules of the hypophysectomized rat. In the present study androsterone definitely increased the weight of the adult dove testis. It therefore appears that androsterone has a rather similar action on the dove and rat testis, and that two previous reports of adverse action of androsterone on the testes of doves and fowl were in error. Bates, Riddle and Lahr (1937) injected a non-crystalline preparation of androsterone (Erugon) into a small group of adult male doves and observed a marked reduction in weight of testes. Morató-Manaro and others (1938) apparently used this same preparation and reported that its inunction led to testis atrophy in cockerels. In both of these cases the observed atrophy of testes was conceivably due to the presence of estrogen in the samples of Erugon.

Desoxycorticosterone acetate and diethylstilbestrol are here found, for the first time, to produce a profound and rapid atrophy of the bird testis. The effectiveness of desoxycorticosterone approaches that of the estrogens. It is far more effective than progesterone, and its action on the dove testis is the opposite of the action of androsterone. Prolonged overdosage of very young fowls with desoxycorticosterone acetate was observed by Selye (1943) to cause fluid retention in the tubules and atrophy of Leydig cells.

The profound atrophy of the germinal tissue in dove testes following treatments with estrone, estradiol benzoate, stilbestrol and desoxycorticosterone acetate presumably results from ability of these steroids to inhibit release of FSH by the pituitary of the male dove. That this is the mechanism through which prolactin produces atrophy of the mature dove testis was shown by Bates, Riddle and Lahr (1937).

SUMMARY

Ten daily injections of desoxycorticosterone acetate, estrone, estradiol benzoate, diethylstilbestrol and prolactin into mature male ring doves resulted in very extensive atrophy of germinal tissues. Testes were thus reduced to 14 to 22 per cent of their pre-treatment weights. The testes of birds treated with progesterone (2.0 mg. daily plus pellet) retained 73 per cent of their pre-treatment weight and showed only slight tissue damage. Cholestenone (3 mg. daily plus pellet) produced no measurable effect on the dove testis. Phenol did not clearly decrease testis weight but it damaged the Leydig cells. In all of three groups (15 birds) treated with androsterone (0.8 to 2 mg. plus pellet) the normal weight of the testes was increased by 23 to 39 per cent. Body weights were increased by treatment with progesterone, desoxycorticosterone and prolactin. Of the several substances tested only estrone had a slightly adverse effect on body weight.

REFERENCES

BATES, R. W., O. RIDDLE AND E. L. LAHR: *Am. J. Physiol.* 119: 610. 1937.
BRENEMAN, W. R.: *Endocrinology.* 21: 503. 1937.
CHU, J. P.: *J. Endocrinol.* 2: 21. 1940.
EMMENS. C. W.: *J. Physiol.* 95: 379. 1939.
LAHR, E. L., O. RIDDLE AND R. W. BATES: *Am. J. Physiol.* 113: 84. 1935.
MOORE, C. R., AND C. F. MORGAN: *Endocrinology.* 30: 990. 1942.
MORATÓ-MANARO, J., A. S. ALBRIEUX AND W. BUÑO: *Arch. Soc. de biol. de Montevideo.* 6: 3. 1935.
MORATÓ-MANARO, J., A. S. ALBRIEUX AND W. BUÑO: *Klin. Wchnschr.* 15: 206. 1938.
NELSON, W. O.: *Anat. Rec.* 67: Suppl. 1, 110. 1936.
PFEIFFER, C. A., AND W. U. GARDNER: *Anat. Rec.* 72: 59. 1938.
RIDDLE, O., AND E. L. LAHR: (in press).
RIDDLE, O., E. L. LAHR AND R. W. BATES: *Am. J. Physiol.* 137: 299. 1942.
SELYE, H.: *J. Morphol.* 73: 401. 1943.
SELYE, H., AND S. ALBERT: *Am. J. M. Sc.* 204: 876. 1942.
SELYE, H., AND S. FRIEDMAN: *Endocrinology.* 28: 129. 1941.
ZONDEK, B.: *Folia Clinica Orient.* 1: 1. 1937.

THE EFFECT OF SEX HORMONES ON THE ANEMIA INDUCED BY HEMORRHAGE IN THE RAT

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MANY INVESTIGATORS have examined the effects of endocrine glands such as the thyroid (Lim, Sarker and Brown, 1922; Kunde, 1926; Hoskins and Sleeper, 1929; Meyer, Thewlis and Rusch, 1940; Crafts, 1941; Vollmer, Gordon and Charipper, 1942), adrenal (Hartman, Green, Bowen and Thorn, 1932; Corey and Britton, 1932; Zwemer and Lyons, 1932; Meyer, Thewlis and Rusch, 1940; Dalton and Masson, 1940; Crafts, 1941, Lewis, 1941), gonads (Jolly, 1909; Kleineberger and Carl, 1912; Adams and Shevket, 1929; Juhn and Domm, 1930; Riddle and Braucher, 1934; Kamenoff, 1937; Steinglass, Gordon and Charipper, 1941; Vollmer and Gordon, 1941; Taber, Davis and Domm, 1943), and anterior pituitary (Meyer, Stewart, Thewlis and Rusch, 1937; Flaks, Himmel and Zlotnick, 1937; Overbeek and Querido, 1938; Querido and Overbeek, 1938; Querido and Overbeek, 1939; Vollmer, Gordon, Levenstein and Charipper, 1939; Vollmer and Gordon, 1941; Crafts, 1941) on the processes of blood formation and destruction. Although there is some conflicting evidence arising from these investigations, the results, in general, seem to indicate that glandular deficiency induces an anemia, and that the administration of certain glandular extracts and hormones tends to correct that anemia.

The evidence that the gonads, anterior pituitary, and other endocrine glands exercise a definite influence on the erythropoietic process appears to be well established. No detailed quantitative experiments, however, have as yet been performed to determine how gonadectomized and hypophysectomized animals respond to an erythropoietic stress such as that produced by bleeding, and whether the sex hormones can influence the recovery from such an anemia. In this connection, the finding that human female blood donors regenerate red blood cells at a slower rate when compared with males (Giffin and Haines, 1923; Martin and Myers, 1934; Fowler and Barer, 1942)

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might be interpreted to mean that the sex hormones play a rôle in this response.

The present investigation is concerned with a study of the effects of castration, hypophysectomy, and sex hormones on the recovery from anemia induced in rats by bleeding. In addition, it was considered advisable to compare the effects of these operations and hormones on the blood picture of the bled rat with those exerted by cobalt, a powerful erythropoietic agent (Waltner and Waltner, 1929; Mascherpa, 1930; Myers, Beard and Barnes, 1931; Orten, Underhill and Lewis, 1932; Kleinberg, 1934; Kleinberg, Gordon and Charipper, 1939).

The present work will include a description and comparison of the red cell counts, hemoglobin contents, and reticulocyte percentages for each series of animals and an analysis of the bone marrow. Preliminary reports on several phases of this work have already appeared (Gordon and Charipper, 1943; Finkelstein, Gordon and Charipper, 1943).

MATERIALS AND METHODS

Five series of a closely inbred strain of adult male and female rats weighing 160–250 grams were used in these experiments. They were fed a balanced mixed diet including fresh lettuce and carrots and were provided with an adequate supply of drinking water. They were maintained in an animal room kept at a constant temperature.

The hypophysectomies, done by a parapharyngeal approach, and the bilateral castrations were both performed under ether anesthesia. The animals in each group were bled by cardiac puncture. Three to 4 cc. of blood were drawn from the hypophysectomized group over a two-day period. The other animals had 4 to 5 cc. of blood drawn at one time with the exception of one series (animals shown in figs. 3 and 4 below) from which 10 cc. of blood were extracted over a two-day period. Subcutaneous injections of the sex hormones[2] and cobalt in the experimental groups were started on the day following bleeding. Treatment was continued until the blood levels returned to normal.

Peripheral blood drawn from the tail was used. Total red cell counts (RBC), hemoglobin (Hb) determinations, and reticulocyte counts were made on the day following bleeding and at weekly intervals.

A Levy-Hausser hemacytometer was employed for the total erythrocyte counts, and each count was made in duplicate. The red cell counts were required to agree within 4%. Reticulocyte counts were made from wet mounts of whole blood in 2% sodium citrate solution, stained with brilliant cresyl blue. One thousand cells were counted in each mount and the reticulocytes were expressed as a percentage of these cells. A Fisher electrohemometer was used for the hemoglobin determinations.

Bone marrow was examined in both the right and left femurs. The bones were fixed in Downey's solution for 24 hours. They were washed in running tap water for 12 hours, decalcified in a solution of equal parts of formic acid

[2] Thanks are extended to Dr. Erwin Schwenk, Schering Corporation, for supplying the estradiol benzoate and testosterone propionate.

and sodium citrate for 24 to 48 hours depending on the size of the bone, and embedded in paraffin. The sections were cut at 5 micra and treated with Kingsley's (1937) stain.

RESULTS

Effects of sex hormones on blood regeneration in bled normal rats. The erythrocyte count of 16 normal male and female rats in this group varied from 8.1–8.7 millions. On the day following the bleeding,

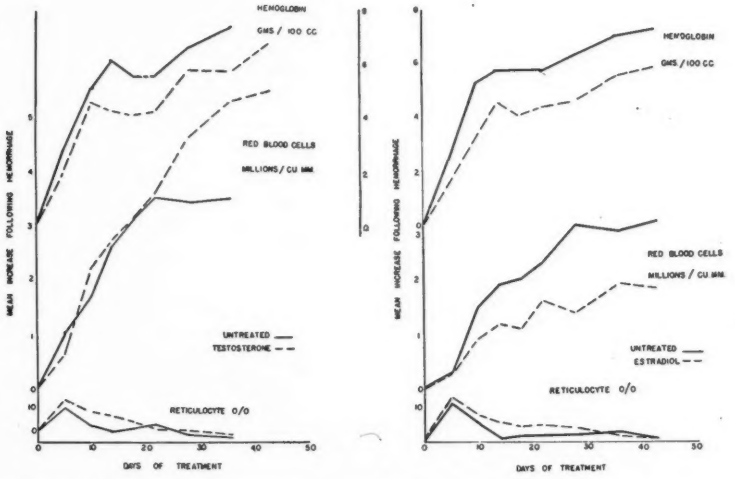

FIG. 1. Effects of testosterone on the red cell counts, hemoglobin contents, and reticulocyte percentages in normal male rats following one bleeding.

FIG. 2. Effects of estradiol on the red cell counts, hemoglobin contents, and reticulocyte percentages in normal female rats following one bleeding.

these values dropped to 5.2–5.6 millions. Four of the bled females received daily injections of gradually increasing doses of estradiol benzoate (20–200 R.U.) over a period of 40 days. Four of the bled males were injected with 1.25–2.5 mg. of testosterone propionate daily for the same period of time. The remaining 4 males and 4 females were given no treatment following the bleeding. Figs. 1 and 2 indicate that normal males regenerated red cells and possibly Hb faster than females and in addition, that male sex hormone accelerated and female sex hormone inhibited the rate of red cell regeneration in bled animals. Both estradiol and testosterone appeared to delay the regeneration of Hb. The reticulocyte rise induced by the hemorrhagic anemia was not significantly altered by either hormone.

A comparison of the effects of testosterone and cobalt on blood regeneration in bled normal rats. Twenty-five normal male and female rats with a RBC of 4.5–4.6 millions on the day following bleeding were

treated as follows: Four males and 4 females were injected with 1.25 mg. testosterone propionate daily for 40 days; 5 females and 4 males were given injections of 2.5 mg. $Co(NO_3)_2 \cdot 6H_2O$ daily for 40 days. Four female and 4 male rats remained untreated. A comparison of Figs. 3 and 4 will indicate that both testosterone and cobalt accelerated red cell regeneration in both sexes. In the male, cobalt appeared

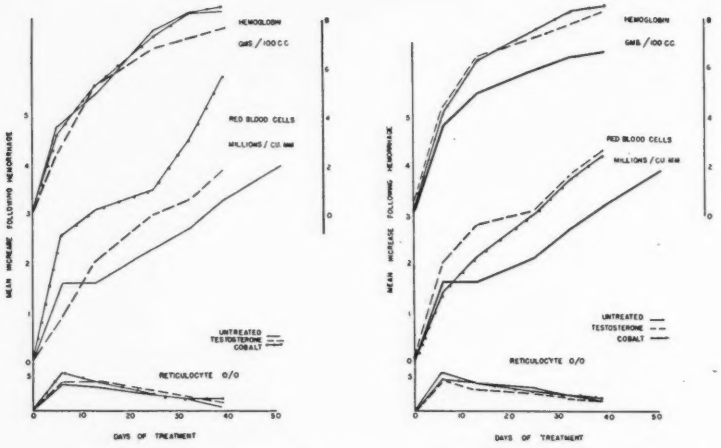

FIG. 3. A comparison of the effects of testosterone and cobalt on the red cell counts, hemoglobin contents, and reticulocyte percentages in normal male rats following 2 successive bleedings.

FIG. 4. A comparison of the effects of testosterone and cobalt on the red cell counts, hemoglobin contents, and reticulocyte percentages in normal female rats following 2 successive bleedings.

to be the more effective of the two, whereas in the female, cobalt and testosterone were approximately equally effective. The Hb response varied somewhat in males and females. In the former, testosterone appeared to inhibit slightly Hb regeneration and cobalt had no effect. In the females, both testosterone and cobalt were found to accelerate Hb regeneration and to about the same extent. In all cases, the reticulocyte response to the bleeding was not affected by hormone or cobalt therapy.

Effects of testosterone on blood regeneration in bled hypophysectomized rats. The erythrocyte counts of 8 representative male hypophysectomized animals varied between 6.1 and 6.7 millions 3 months following the operation, at which time they were bled. On the day following the second bleeding, the RBC of these rats had dropped to 4.0–4.1 millions. Four of the rats received daily injections of 1.25 mg. testosterone propionate and the remaining 4 were allowed to continue untreated. Fig. 5 indicates that hypophysectomized rats were capable

of regenerating red cells following bleeding and that testosterone definitely accelerated recovery from the anemia. This was reflected not only in the behavior of the RBC but also in the Hb. It can also be observed that hypophysectomized rats showed a reticulocytosis following bleeding as marked as that revealed by bled normal rats; the extent of this reticulocyte response, however, was not altered by testosterone treatment.

Effects of testosterone, cobalt and the combination on blood regeneration in castrated rats. The normal male rat has a RBC somewhat higher than that of the female (Steinglass, Gordon and Charipper, 1941).

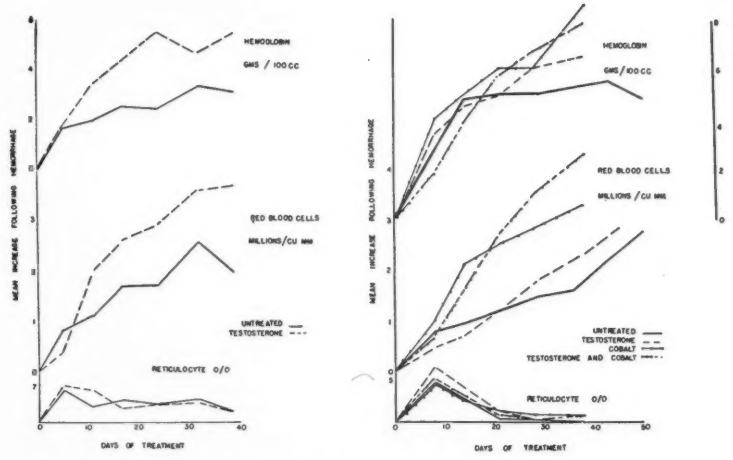

FIG. 5. Effects of testosterone on the red cell counts, hemoglobin contents, and reticulocyte percentages in hypophysectomized male rats following 2 successive bleedings.

FIG. 6. A comparison of the effects of testosterone, cobalt and the combination on the red cell counts, hemoglobin contents, and reticulocyte percentages in castrated male rats following one bleeding.

Seventeen castrated male rats and 13 castrated females were bled one week following the operation. On the day following the bleeding, the RBC dropped to 4.5–4.8 millions. Four females and 5 male animals were then injected daily with 1.25 mg. testosterone propionate; four females and 5 males were similarly treated with 1.25 mg. $Co(NO)_3)_2.6H_2O$. Five females and 7 males were allowed to continue untreated.

Observation of Figs 6 and 7 will show that red cell and Hb regeneration were more rapid in the bled castrated females than in the bled castrated males. Testosterone accelerated red cell regeneration in both castrated males and females. It caused a more rapid replacement of Hb in the castrated males but not in the castrated females.

Cobalt also speeded red cell and Hb formation in both castrated males and females. The combination of testosterone and cobalt was most effective on red cell regeneration. Its influence on Hb regenera-

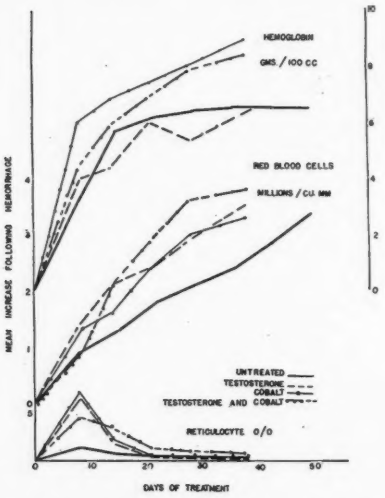

FIG. 7. A comparison of the effects of testosterone, cobalt, and the combination on the red cell counts, hemoglobin contents, and reticulocyte percentages in castrated female rats following one bleeding.

tion, however, was not greater than that induced by cobalt alone. Reticulocyte behavior was no different in any of the experimental groups.

Bone marrow description. A reduction in the quantity of hemogenic tissue and the presence of extensive vacuolization are consistent fea-

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DESCRIPTION OF FIGS. 8–13

FIGURES 8–13 represent transverse sections of rat femoral bone marrow, fixed in Downey's solution, decalcified in formic acid and sodium citrate, and treated with Kingsley's stain. ×240.

FIG. 8. Bled hypophysectomized male rat treated with testosterone for 40 days. There is partial repair of the hypophysectomy hypoplasia.

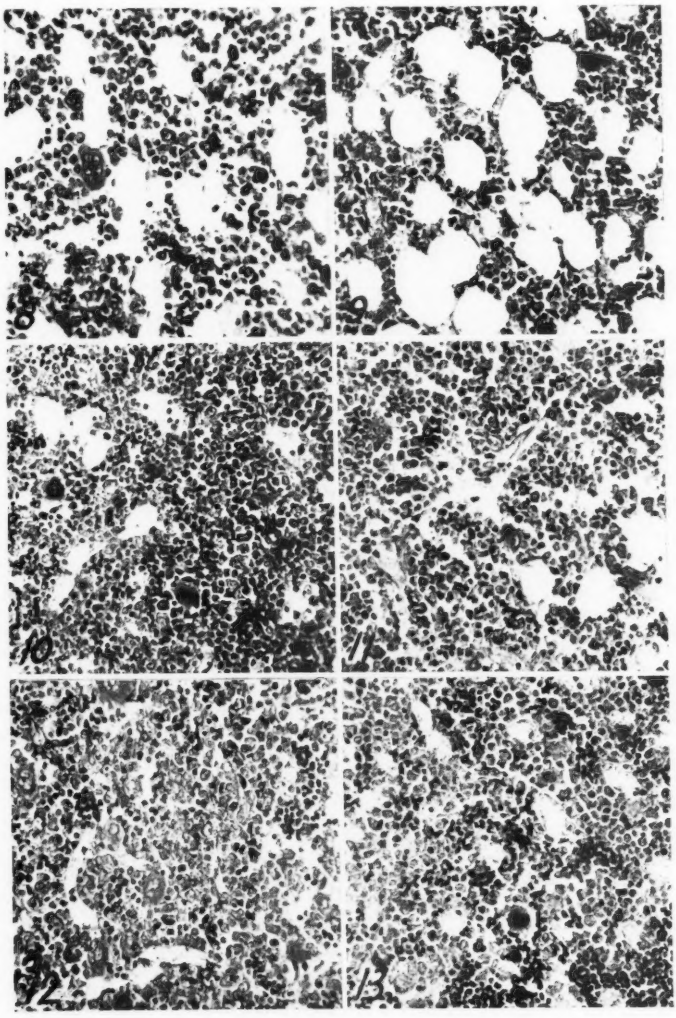
FIG. 9. Bled castrated male rat given no treatment. Vacuolization and slight hypoplasia are apparent 40 days after bleeding.

FIG. 10. Bled castrated female rat given no treatment. Little vacuolization and some hyperplasia of the erythrogenic elements are visible 40 days after bleeding. Contrast with Fig. 9.

FIG. 11. Bled castrated male rat treated with testosterone for 40 days. Partial repair of the castration hypoplasia is observed. Compare with Fig. 9.

FIG. 12. Bled castrated male rat treated with cobalt for 40 days. Considerable erythrogenic proliferation is observed, greater than that seen in Fig. 11.

FIG. 13. Bled castrated male rat treated with both testosterone and cobalt for 40 days. Complete repair noted. Several areas are intensely hyperplastic.



FIGS. 8–13

tures of the bone marrow of the hypophysectomized rat (Vollmer, Gordon, Levenstein and Charipper, 1939). Although the stimulus of bleeding tends to activate the marrow, the hypoplasia in the hypophysectomized animals' marrow is again apparent 40 days after the removal of the blood. Treatment of the bled hypophysectomized rats with testosterone tends to prevent this hypoplasia (Fig. 8). The numbers of erythroblasts and normoblasts show a definite increase;

vacuolization, however, is not completely repaired. Megakaryocytes appear in increased numbers.

The bone marrows of untreated castrated male rats, 43 days after bleeding are hypoplastic (Fig. 9) although not to such an extent as the marrows of castrated male animals not subjected to bleeding. The hypoplasia apparent in the castrated male rat is not to be seen in the castrated female subjected to bleeding (Fig. 10). In fact, in the latter, the condition resembles that found in the bled normal male rat. Bled castrated male rats treated with testosterone display a stimulated bone marrow with, however, the persistence of some vacuolization (Fig. 11). Injections of cobalt completely overcome the hypoplasia of the castrated male rats' bone marrow. Erythrogenic activity is greatly increased as evidenced by the appearance of increased numbers of erythroblastic elements (Fig. 12). The greatest extent of hyperplasia in the castrated male rats' marrow occurs after the injection of both testosterone and cobalt (Fig. 13). Here greatly increased numbers of erythroblasts and normoblasts are visible; mitotic activity of these elements is very marked.

DISCUSSION

The present studies indicate quite clearly that the sex hormones are concerned with recovery from the anemia induced by bleeding in the rat. This statement is based on the following evidence presented in the results: first, bled normal male rats regenerate red cells more rapidly than bled normal females; secondly, recovery from anemia is slower in castrated males than in castrated females, and lastly, injections of testosterone accelerate and estradiol inhibit rate of red cell regeneration in hemorrhagic anemia. These findings along with the bone marrow studies further support the hypothesis that androgens stimulate and estrogens depress erythrogenic activity (Steinglass, Gordon and Charipper, 1941; Vollmer and Gordon, 1941).

The data show that the hypophysectomized rat responds to the stimulus of bleeding by an outpouring of reticulocytes about equal in quantity to that evoked in the normal rat. Red cells and Hb continue to be regenerated in such animals until the values characteristic of the post-hypophysectomy anemia are attained. On the other hand, Stewart, Greep and Meyer (1935) and Meyer, Thewlis and Rusch (1940) have reported no significant increase in RBC, Hb or reticulocytes in hypophysectomized rats subjected to reduced atmospheric pressures. This difference in results is somewhat difficult to understand in view of the commonly accepted opinion that bleeding and lowered barometric pressures affect the bone marrow in a similar manner. It would be worth while to compare further the effects of bleeding and exposure to lowered oxygen tensions in endocrine-deficient animals. It is, however, quite clear from the data that testosterone will accelerate recovery from the marked anemia induced by

hemorrhage in the hypophysectomized rat. This is reflected in both the behavior of the RBC and Hb of such animals.

One of the most interesting findings in the present study is the synergism exhibited between testosterone and cobalt on the RBC response. It has been shown that cobalt, by itself, is a powerful erythropoietic stimulus in inducing the recovery from hemorrhagic anemia in the rabbit (Kleinberg, Gordon and Charipper, 1939) and in the rat. If testosterone is administered along with the cobalt, the effects on the red cell response and marrow histology are even more striking. It would be of interest to determine whether this combination is of benefit in other types of artificially induced anemias and in clinical blood deficiencies. In this connection, the interesting report of Glass (1943) might be cited. He showed that treatment with thyroid, liver extract, and iron was without effect on the course of a hyperchromic macrocytic anemia in a patient suffering from myxedema. When, however, testosterone was coupled with thyroid, the recovery from the anemia was rapid and complete.

The results obtained with testosterone would tend to indicate that although this hormone serves as a stimulus to erythrogenic proliferation, it does not necessarily accelerate Hb regeneration. It may actually delay the formation of Hb as it does in bled normal male and castrated female rats. On the other hand, testosterone accelerates Hb regeneration in bled normal females, castrated males, and hypophysectomized male rats. Cobalt, in the dosages employed, appears to stimulate Hb regeneration more effectively than testosterone. It would be profitable to study the effects of agents known to accelerate Hb formation in testosterone-treated animals.

Too much reliance cannot be placed on the significance of the reticulocyte changes occurring in the animals studied in this report. In every case, reticulocytosis is observed during the week following bleeding. The post-bleeding reticulocyte levels or the time of attainment of normal reticulocyte counts, however, do not appear to be significantly altered by any of the hormone treatments. It has been repeatedly demonstrated, moreover, that alterations in the red cell counts of animals cannot necessarily be correlated with the behavior of the reticulocytes. Meyer et al. (1940) have found, for example, that cevitamic acid or sodium bicarbonate can induce reticulocytosis in the hypophysectomized rat without affecting the anemia. It is also quite possible that an increase in the red cell count which is slow and protracted may not necessarily be accompanied by a significant reticulocytosis. As pointed out in a previous report (Vollmer, Gordon and Charipper, 1942), we have come to judge an increase in reticulocytes as important only when it precedes an actual rise in red cell count such as occurs after bleeding (Gordon, 1934) or during exposure to low atmospheric pressures (Gordon and Kleinberg, 1937).

It cannot be argued that the alterations in red cell counts observed

in the hormone treated animals are due to hemoconcentration or hemodilution effects since it is not possible to relate quantitatively the Hb changes to those occurring in erythrocyte numbers. The red cell behavior in the treated rats, on the other hand, is best correlated with the marrow histology which is the most reliable indication of the fact that the hormones are affecting the erythrogenic process itself. The precise mechanism of action of the hormones in erythropoiesis, however, still remains to be determined (Vollmer, Gordon and Charipper, 1942).

SUMMARY

Normal male rats, subjected to bleeding show a greater rate of red cell regeneration than bled normal females; in such animals, male sex hormone shortens and female sex hormone delays the time of attainment of normal red cell values. Red cell regeneration is more rapid in bled castrated females than in bled castrated males; male sex hormone accelerates red cell regeneration in both these types of animals. Injections of male sex hormone into bled hypophysectomized rats shortens the time of recovery from the anemia. The combination of testosterone and cobalt is more effective in speeding red cell regeneration in castrated rats than either agent alone.

Proportionate increases in Hb concentration do not necessarily occur along with increases in red cell count in the experimental animals treated with the sex hormones; testosterone tends to inhibit Hb regeneration in bled normal males and castrated females whereas it accelerates Hb formation in bled normal females, castrated males, and hypophysectomized male rats. None of the hormone or cobalt treatments is found to affect the values of the post-hemorrhagic reticulocytosis, nor do they alter the time of attainment of normal reticulocyte counts in any of the bled experimental animals. Bone marrow studies lend further support to the contention that the sex hormones play a normal rôle in the process of erythrogenesis and that male sex hormone aids in recovery from hemorrhagic anemia by virtue of its erythropoietic effect.

REFERENCES

Adams, A. E., and F. Shevket: *Physiol. Zool.* 2: 181. 1929.
Corey, E. L., and S. W. Britton: *Am. J. Physiol.* 102: 699. 1932.
Crafts, R. C.: *Endocrinology.* 29: 596. 1941.
Dalton, A. J., and G. Masson: *Proc. Soc. Exper. Biol. & Med.* 43: 370. 1940.
Finkelstein, G., A. S. Gordon and H. A. Charipper: *Anat. Rec.* 87: 441. 1943.
Flaks, J., I. Himmel and A. Zlotnick: *Presse med.* 45: 1261. 1937.
Fowler, W. M., and A. P. Barer: *J.A.M.A.* 118: 421. 1942.
Giffin, H. Z., and S. F. Haines: *J.A.M.A.* 81: 532. 1923.
Glass, S. J.: *J. Clin. Endocrinol.* 3: 421. 1943.
Gordon, A. S.: *Proc. Soc. Exper. Biol. & Med.* 31: 563. 1934.
Gordon, A. S., and W. Kleinberg: *Proc. Soc. Exper. Biol. & Med.* 37: 507. 1937.
Gordon, A. S., and H. A. Charipper: *Anat. Rec.* 85: 24. 1943.
Hartman, F. A., C. W. Green, B. P. Bowen and G. W. Thorn: *J.A.M.A.* 99: 1478. 1932.

HOSKINS, R. G., AND F. J. SLEEPER: *Endokrinologie.* 5: 89. 1929.
JOLLY, J.: *Compt. rend. Soc. de biol.* 66: 136. 1909.
JUHN, M., and L. V. DOMM: *Am. J. Physiol.* 94: 656. 1930.
KAMENOFF, R. J.: *Proc. Soc. Exper. Biol. & Med.* 36: 411. 1937.
KINGSLEY, D. M.: *Folia haemat.* 57: 87. 1937.
KLEINBERG, W.: *Am. J. Physiol.* 108: 545. 1934.
KLEINBERG, W., A. S. GORDON AND H. A. CHARIPPER: *Proc. Soc. Exper. Biol. & Med.* 42: 119. 1939.
KLIENEBERGER, C., AND W. CARL: Die Blut-Morphologie der Laboratorums-Tiere. J. A. Barth. Leipzig. 1912.
KUNDE, M. M.: *Am. J. Physiol.* 76: 225. 1926.
LEWIS, L. A.: *Endocrinology* 28: 821. 1941.
LIM, R. K. S., B. B. SARKER AND J. P. H. GRAHAM BROWN: *J. Path. & Bact.* 23: 228. 1922.
MARTIN, J. W., AND J. L. MYERS: *J. Lab. & Clin. Med.* 20: 593. 1934–35.
MASCHERPA, P.: *Arch. ital. de biol.* 82: 112. 1930.
MEYER, O. O., G. E. STEWART, E. W. THEWLIS AND H. P. RUSCH: *Folia haemat.* 57: 99. 1937.
MEYER, O. O., E. W. THEWLIS AND H. P. RUSCH: *Endocrinology.* 27: 932. 1940.
MYERS, V. C., H. H. BEARD AND B. O. BARNES: *J. Biol. Chem.* 94: 117. 1931.
ORTEN, J. M., F. A. UNDERHILL AND R. C. LEWIS: *J. Biol. Chem.* 96: 1. 1932.
OVERBEEK, G. A., AND A. QUERIDO: *Arch. internat. de pharmacodyn. et de therap.* 60: 107. 1938.
QUERIDO. A., AND G. A. OVERBEEK: *Arch. internat. de pharmacodyn. et de therap.* 59: 370. 1938; 61: 475. 1939.
RIDDLE, O., AND P. F. BRAUCHER: *Am. J. Physiol.* 106: 554. 1934.
STEINGLASS, P., A. S. GORDON AND H. A. CHARIPPER: *Proc. Soc. Exper. Biol. & Med.* 48: 169. 1941.
STEWART, G. E., R. O. GREEP AND O. O. MEYER: *Proc. Soc. Exper. Biol. & Med.* 33: 112. 1935.
TABER, E. D., E. DAVIS AND L. V. DOMM: *Am. J. Physiol.* 138: 479. 1943.
VOLLMER, E. P., A. S. GORDON, I. LEVENSTEIN AND H. A. CHARIPPER: *Endocrinology* 25: 970. 1939.
VOLLMER, E. P., AND A. S. GORDON: *Endocrinology.* 29: 828. 1941.
VOLLMER, E. P., A. S. GORDON AND H. A. CHARIPPER: *Endocrinology.* 31: 619. 1942.
WALTNER, K., AND K. WALTNER: *Klin. Wchnschr.* 5: 2382. 1929.
ZWEMER, R. L., AND C. LYONS: *Am. J. Physiol.* 36: 545. 1928.

NOTES AND COMMENTS

EFFECT OF THIOURACIL ON THE PLUMAGE OF THE BROWN LEGHORN CAPON[1]

IT HAS BEEN shown conclusively in recent years that thiourea and certain of its derivatives effect hyperplasia and hypertrophy of the thyroid gland (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell and Tyslowitz, 1943; Astwood, 1943; Kennedy, 1942; Richter and Clisby, 1942), and that there is a concommitant depression of thyroid activity (MacKenzie and MacKenzie, 1943; Astwood, Sullivan, Bissell and Tyslowitz, 1943).

The greater number of these investigations were carried out in rats but other mammals such as the mouse and the dog gave similar reactions when treated with goitrogenic drugs. In chickens fed sulfaguanidine MacKenzie and MacKenzie (1943) reported no inhibition of the thyroid gland; however Mixner, Reinecke and Turner (1944) found an hypertrophy in chicks fed thiouracil and thiourea.

It is now shown that the administration of thiouracil[2] to the Brown Leghorn capon causes alterations of structure and of pigmentation in the feathering which are comparable to those initiated by thyroidectomy. Thiouracil was fed to ten Brown Leghorn capons as an 0.5 per cent suspension in tap water and added to the dry feed to form a wet mash. Each bird consumed approximately 0.4 gm. of thiouracil per day. This dosage caused hitherto black feathers or feather segments to be replaced by red and simultaneously provoked a reduction of barbulation so that originally solid feathers became lacy or fringed. These same responses in the plumage have been found to follow surgical removal of the thyroid gland in the male of this breed of fowl (Greenwood and Blyth, 1929; Domm, 1942).

The course of the reaction to thiouracil has been followed by several procedures. Astwood and Bissell (1944) measured the disappearance of iodine from the thyroids of rats treated with thiouracil and determined a definite decrease after 24 hours; this decrease continued to the fifth day at which time the concentration had fallen to one-thirtieth of the normal value, a level at which it remained for the duration of the experiment. Upon cessation of treatment iodine rapidly returned to the gland but the concentration was not up to normal at the end of a week. In chicks one or two days of age, fed thiouracil for two weeks, Mixner, Reinecke and Turner (1944) found a significant weight increase of the thyroid on the fourth day and the maximum was achieved on the twelfth day.

In the capon the sequence of the response to thiouracil was followed by a

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[1] Scientific paper No. A87, Contribution No. 1946, of the Maryland Agricultural Experiment Station (Department of Poultry Husbandry).

[2] The thiouracil used was obtained through the kindness of Dr. Mark Welsh, Lederle Laboratories, Inc.

weekly examination of regenerating breast feather germs in regions which had been previously deplumed (Methods: Juhn and Gustavson, 1930). The first modification from black to red was found approximately nineteen days after the commencement of feeding and red pigment continued to be laid down approximately one week after administration of the drug was discontinued. As the deposition of red pigment in the feather germ is taken to reflect a depletion of thyroid hormone and the reappearance of the black pigment is considered an indication of return to normal of the thyroid, the time intervals given in these pigment changes differ somewhat from those shown by other measures; the time of onset of effect being considerably slower.

Summary. Thiouracil fed in daily dosages of approximately 0.4 gm. to ten adult Brown Leghorn capons was found to cause the replacement of black feather segments by red pigment and to effect a reduction in barbulation. These changes are similar to those obtained following thyroidectomy in this breed of fowl.

REFERENCES

ASTWOOD, E. B.: *J. Pharmacol. & Exper. Therap.* 78: 79. 1943.
ASTWOOD, E. B., J. SULLIVAN, A. BISSELL AND R. TYSLOWITZ: *Endocrinology.* 32: 210. 1943.
ASTWOOD, E. B., AND A. BISSELL: *Endocrinology.* 34: 282. 1944.
DOMM, L. V.: *Anat. Rec.* 84: 79. 1942.
GREENWOOD, A. W., AND J. S. S. BLYTH: *Proc. Roy. Soc. Edinburgh.* 49: 313. 1929.
JUHN, M., AND R. G. GUSTAVSON: *Proc. Soc. Exper. Biol. & Med.* 27: 747. 1930.
MACKENZIE, C. G., AND J. B. MACKENZIE: *Endocrinology.* 32: 185. 1943.
MIXNER, J. P., E. P. REINECKE AND C. W. TURNER: *Endocrinology.* 34: 168. 1944.
RICHTER, C. P., AND K. H. CLISBY: *Arch. Path.* 33: 46. 1942.

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ASSOCIATION NOTICE

At the Twenty-seventh Annual Meeting of the Association in Chicago, June 12 and 13, 1944, the following revision of the By-Laws[1] of the Society was recommended by the Council and was adopted by the members at the annual business session.

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS BY-LAWS

Article I—Name and Object

The name and object of this Association shall be as set forth in the Articles of Incorporation. The organization shall be conducted for scientific purposes, for the advancement and promulgation of knowledge regarding the internal secretions and for the facilitation of personal relationship among investigators in the subject of endocrinology.

Article II—Membership

Section 1. The Association shall consist of active members only. Of these, there shall be two classes, annual members and life members.

Section 2. Eligibility: Any qualified physician or biologist in good professional standing shall be eligible for nomination to annual membership. Annual members of high professional standing may, upon invitation by the Council, become life members upon payment of one hundred dollars, which sum shall be in lieu of annual membership assessments.

Section 3. Nomination and Election: Nominations for membership shall be made and seconded by members of the Association on blanks furnished by the Secretary, and shall be submitted to the Council. The Council shall determine eligibility and shall vote by ballot upon nominations. A majority of votes cast shall constitute election.

Section 4. Resignation, Forfeiture and Expulsion: The resignation of any member in good standing may be accepted at any annual meeting of the Council, providing his dues have been paid in full for the current year.

Any member whose annual assessment is in arrears one month after the sending of a second notice to his last known address shall automatically

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[1] The Constitution of the Association comprises the Articles of Incorporation, as set forth in the certificate recorded with the Secretary of State of the State of Delaware on January 31, 1918.

These Articles of Incorporation grant broad powers to the Association; give it perpetual existence; exempt entirely the private property of members for the payment of corporate debts; specify the general qualifications for membership; and authorize the Society to make and alter By-Laws for the regulation of its activities.

forfeit his membership. Delinquent members may be reinstated by the Council, providing all indebtedness to the Association is discharged.

Upon the recommendation of the Council, any member may be expelled by a majority vote of the members present at an annual meeting of the Association, provided that a copy of the charges against the member in question and a notice of hearing thereon by the Council shall have been given him at least thirty days before the annual meeting.

Article III—Meetings and Quorum

Section 1. Annual: The annual meeting of the Association shall be held at such time and place as shall be determined by the Council.

Section 2. Special: Regional branches of the Association may be authorized by the Council, upon petition by ten members. These may hold special scientific meetings as desired and elect their own officers.

Section 3. Quorum: Fifteen members shall constitute a quorum for the transaction of business at any annual meeting.

Section 4. Council Meetings: At least one meeting of the Council shall be held at each annual meeting of the Association. In the interval between annual meetings the President may on his own volition or at the request of two members submit questions by mail to the members of the Council for their consideration and decision.

Article IV—Officials

Section 1. Officers: The officers shall be a President, a President-Elect, a Vice-President and a Secretary-Treasurer, who shall be elected annually for a term of one year by the members of the Association. The officers shall enter upon their duties at the close of the annual meeting at which they are elected. The powers and duties of the officers shall be such as usually devolve upon their respective positions.

Section 2. Council: The officers so elected and nine additional members, three of whom shall be elected at each annual meeting, shall constitute the Board of Directors of the Corporation and shall be known as the Council. The term of office of members of the Council shall be three years. The President of the Association shall be ex-officio the Chairman of the Council. The Secretary-Treasurer shall be ex-officio the Secretary of the Council.

Section 3. Powers: The general management of the Association shall be vested in the Council. It shall regularly perform the ordinary duties of a board of directors of a corporation and shall possess all the powers conferred upon the directors by the Charter of Incorporation. The Council shall have power at any time to appoint any committee it may deem necessary and to delegate whatever powers necessary to such committees.

Article V—Nomination of Officers

Section 1. Nominating Committee: The Nominating Committee shall consist of three members appointed for the ensuing year by the newly-elected President and accepted by the Council. The President shall designate one member to be Chairman of the Nominating Committee.

Section 2. Nomination of Officials: A) The Nominating Committee shall make at least one nomination for each of the four offices and for each of the positions on the Council to be filled by vote of the members. Any member of the Association may submit nominations to the Nominating Committee

for its consideration. B) The nominations of the Nominating Committee shall be transmitted to the Secretary at least sixty days before the annual meeting at which they are to be considered. C) The Secretary shall send to each member, at least four weeks before the annual meeting, a printed ballot containing the list of nominees and space for such additional names as the member wishes to propose, and at the same time shall notify the members that they may vote by mail by returning the marked ballot to the Secretary before the beginning of the business session at which the elections are to take place.

Section 3. Elections: At the annual meeting the Secretary shall present to the Tellers, appointed by the President, the ballots submitted by the members. The Tellers shall count the votes and announce the results at the business session. A majority of votes cast shall be necessary to elect an official. In case no candidate shall have received a majority of votes, the office in question shall be filled by majority vote of those members present at the business session.

Section 4. Filling of Vacancies: The Council shall fill all vacancies in elective positions except such as occur at an annual meeting of the Association.

Article VI—Financial

Section 1. Dues: The dues shall be the annual cost of subscription to Endocrinology for members, which shall be determined by majority vote at the annual meeting, upon recommendation by the Council.

Section 2. Expenditures: No expenditures from the general funds of the Association except those required in the performance of the ordinary duties of officers shall be made except by vote of the Society or the Council.

Article VII—Publications

Section 1. The official organs of publication of the Association shall be the bulletin Endocrinology and such other periodicals or books as from time to time the Association may authorize. Ownership of these shall be vested in the Association.

Article VIII—Papers at Scientific Sessions

The President and Secretary shall be authorized to arrange programs for the scientific sessions at the annual meeting. The manner of selection of papers to be presented and the time allowed for presentation shall be determined by the Council.

Article IX—Changes in By-Laws

Section 1. These By-Laws, after having been approved by the Council and adopted by the Association, may be amended at any annual meeting as hereinafter provided.

Proposed amendments to the By-Laws must be sent to the Secretary at least four weeks before the date of the annual meeting at which they are to be considered. The Secretary shall promptly transmit them to the members of the Council. At the annual meeting the Council shall consider the proposed amendments and make recommendations to the members at the business meeting of the Association. A majority of the votes of members at the business session of the annual meeting shall be necessary for adoption of an amendment.